BIOMONITORING OF ORGANIC COMPOUNDS IN DRINKING WATER

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The Honourable
Keith C. Norton, Q.C.,
Minister

Gérard J. M. Raymond
Deputy Minister

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By:
INTERNATIONAL ENVIRONMENTAL CONSULTANTS
DUNDAS STREET WEST,
ISLINGTON, TORONTO, ONTARIO

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D. G. Langley
Project Principal

J. Fitchko
Project Manager

D. H. Stendahl per
Project Scientist

PROJECT: 2076.1

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1.0 SUMMARY

This report presents the results of research undertaken by IEC International Environmental Consultants Ltd. on biological monitoring of organic compounds in drinking water in Ontario. The research was conducted from 1979 to 1981 for the Ontario Ministry of the Environment and was financed by the Provincial Lottery Fund.

Water contains a variety of natural and synthetic organic compounds, many of which are found in minute concentrations (parts per trillion). Some of these compounds may pose a potential hazard to the public, based on previous toxicological research. The bioaccumulation of persistent organic compounds such as DDT and PCB's has been of concern for the last two decades. However, since their persistence was first recognized, many other organic compounds have also been demonstrated to bioaccumulate.

The objective of the study was to assess the feasibility of using biological methods to monitor levels of organic compounds in raw water supplied to municipal drinking water systems. Two methods were assessed: bioaccumulation and fish physiography. Bioaccumulation has long-term monitoring and abatement applications whereas a fish physiograph may serve as an alarm indicator of sudden increases in organic levels.

Fish were used to accumulate organics potentially available in raw water supplied by monitoring compounds which bioaccumulate and might pose potential health hazards. Bioaccumulation was also investigated to distinguish natural organic compounds in water from those originating from municipal and industrial discharges. Furthermore, the application of bioaccumulation to identify compounds below chemical analysis detection limits was assessed.

The second method utilizing fish physiography consisted of experiments to assess the feasibility of monitoring alterations in fish respiratory behaviour to detect possible elevated levels of organic compounds in water

supplies. This phase of research was undertaken as there is a need for more reliable and faster methods to monitor potentially hazardous organic compounds in public water supplies. For example, if a slug of a potential contaminant reached the intake of a public water supply, present chemical analysis procedures would not detect the compound until sometime after it had entered the drinking water distribution network. This would occur because of the time required to perform the chemical assay. However, fish could monitor the water continuously, integrating the influences of all chemical substances.

The study area selected for this research was the Grand River basin, the largest watershed in Southern Ontario. Brantford was selected as the experimental site for both the fish physiograph and bioaccumulation studies since the city is dependent on the Grand River as a drinking water source and municipal and industrial outfalls occur upstream of the Brantford water treatment plant intake. The control site for the bioaccumulation study was located at Shades Mill on a small tributary which drains an undeveloped portion of the watershed upstream from Brantford.

Bioaccumulation

The bioaccumulation experiments exposed fish to river water for twelve weeks. Fish tissue and water samples were then chemically analyzed. The results showed that the concentration of organic compounds in the Brantford and Shades Mill drinking water supplies were within Federal Health and Welfare drinking water objectives and did not represent a public health hazard. The findings are discussed more fully below.

Twenty-two organic compounds were identified in the study, all levels close to the chemical analysis detection limits. The detection limits were in the parts per trillion range in water and parts per billion range in fish tissue samples. In spite of the greater sensitivity of water analysis, one third more compounds were measured in fish tissue than identified in water samples which were extracted using a polymer resin. In fact, nine of the twelve

compounds which bioaccumulated in fish were not detected in water. Over the twelve week exposure period, fish accumulated residues of OC pesticides, chlorobenzenes, tetrachlorophenol, PCB and several PAH's. A few compounds detected only in water samples were either non bioaccumulative (eg. atrazine) or probably absorbed to organic particles and unavailable for accumulation (eg. chlordane). Therefore, bioaccumulation not only enabled the detection of trace organics but also established their environmental significance based on residue-forming potential, reactivity and stability.

In spite of the low organic concentrations in Grand River water, the levels and compounds identified in fish tissue at the developed and undeveloped sites clearly demonstrated differences in organic inputs at the two locations on the river basin. By exposure week 12, fish at the site located downstream of treated industrial and municipal discharge areas showed higher levels for 13 of the 15 compounds identified, 5 of which were not detected in fish at the upstream reference site.

A number of factors may influence bioaccumulation in fish and the holding of hatchery fish of uniform size, age and condition in aquaria receiving aerated, temperature-controlled water at a constant flow rate was essential to demonstrate differences in organic content of the water at the two sites on the river. Moreover, the use of a semi-purified fish food formulation was necessary to reduce the possibility of trace organic uptake from the food.

As the fish exposure progressed, the number of compounds demonstrated to bioaccumulate increased. Longer fish exposure may have increased bioaccumulation of the more lipophilic and stable organics. Thus, bioaccumulation identifies the classes of compounds of environmental significance.

The feasibility of using bioaccumulation in fish to monitor organics in drinking water supplies at concentrations below detection limits of conventional water analyses was demonstrated in this study. Fish tissue residues

can thus provide a sensitive measure of public exposure to organics and may be included in future monitoring programs established to protect the public. Except for the addition of some clean up steps, fish tissue analysis was carried out with the same ease and cost as water analysis.

Three biomonitoring approaches are available to investigate potential organic contamination of drinking water.

1. Indigenous fish sampling can be used when prolonged exposure to organics in water and food is of interest. The collection and analysis of resident fish would provide further information on microorganic concentrations in the river system under investigation.

2. In situ biomonitoring (caged fish) could be used in areas that are suspected to be contaminated to confirm source and identity of organics.

3. On site biomonitoring under controlled experimental conditions permits the detection of trace concentrations of organics and enables comparison of different locations. IEC's research successfully used this approach which enabled demonstration of differences in organic inputs at the two experimental sites.

Fish Physiography

IEC evaluated all available information on fish physiography. The investigation included seven fish physiograph systems which had been developed for a variety of laboratory research applications. None of the previous physiograph applications had been used on drinking water and none had been used in Canada. IEC's research showed that fish physiography could be used as a reliable field monitor. The system developed by IEC optimized the features of the other systems for Canadian field application. This research will facilitate the use of fish physiography by other scientists in their investigations. The study recommends that further research should be undertaken to computer automate the system.

In addition to the development of a practical field physiograph system, several experiments were conducted measuring fish respiratory activity. The results showed that organic chemical concentrations required to elicit a respiratory response by fish were several orders of magnitude higher than organic concentrations found in the drinking water supply at Brantford. Fish did not alter their respiratory activity throughout the monitoring period. However, fish respiration may be used to monitor organics if ambient concentrations are significantly higher than levels presently measured in the Grand River.

Dose response physiograph experiments were undertaken using phenol, zinc and ammonia. They showed that alterations in ventilation and cough rate occurred within several hours at 10% to 50% of acutely lethal levels (96 h LC50). In addition, ventilation amplitude occurred at acutely lethal levels and following severe elevations in concentration. Different compounds evoked different fish respiratory responses. Phenol and zinc, which act directly on the gill membrane, affected cough and ventilation rate while ammonia, which interferes with gill physiology, did not stimulate a cough response but altered the pattern of ventilation.

The research demonstrated that fish physiograph systems may be operated to monitor chemical slugs or spills at sub-acute levels and for monitoring the toxicity of effluent discharges. Future research should consider automating the system for continuous monitoring by adding a computer to the physiograph system.

In summary, this report evaluates two methods of monitoring drinking water supply. Body burden accumulation of organics was demonstrated in fish bioaccumulation experiments. Fish ventilatory response using a fish physiograph was used to measure organics in the Brantford raw drinking water supply. The concentrations in untreated Grand River water did not alter fish respiratory activity.

2.0 INTRODUCTION

2.1 BACKGROUND

Raw water supplied to municipal drinking water systems contains a variety of natural and synthetic organic compounds. Recent advances in analytical technology have enabled the measurement of many compounds previously unidentified in water. Some of these organics which are found in minute concentrations (parts per trillion) may pose a hazard to public health. Moreover, organics may result in taste and odour problems, tainting of fish and toxicity to aquatic life.

In order to minimize introduction of man-made organics to water, wastes from municipal and industrial sources are treated prior to release into receiving waters. However, small amounts of synthetic organics may pass unchanged through the treatment process. As a result, monitoring of rivers at points of treated effluent discharges and at areas of water use is needed to establish organic inputs to drinking water treatment plants. Furthermore, increased demands and multiple use of water has intensified the need for monitoring.

At present, water quality monitoring is performed principally by physical and chemical methods. These methods do not provide continuous surveillance as sampling is infrequent. Also, technical and economic considerations limit the number of compounds measured. Thus, compounds which may be hazardous in water may occur undetected and the full potential of these materials to cause harm has not been established; and full toxic interaction between compounds has not be assessed.

In contrast, monitoring methods using living organisms can assess toxicity of substances in water. The organisms maintain a continuous presence in water and thus integrate the influences of all substances in their environment. This monitoring provides a direct measure of the impacts of water quality on a living organism and provides an excellent monitoring technique. This approach, termed "biological monitoring". can be used to detect toxic organic compounds and their effects and thus monitor quality of drinking water supplies.

IEC International Environmental Consultants Ltd. was retained by the Ontario Ministry of the Environment (MOE) to conduct a state-of-the-art research study to biomonitor organic compounds in drinking water supplies using combined techniques of fish physiography and bioaccumulation monitoring.

2.2 STUDY OBJECTIVES

IEC selected the biological monitoring methods of fish physiography and bioaccumulation to fulfill the study objectives outlined by the MOE which were:

1. To identify organic compounds, if any, which are present in raw drinking water and are bioaccumulative in fish;

2. To distinguish naturally occurring organic compounds in raw drinking water from those originating from anthropogenic sources, eg. municipal/ industrial effluent discharges;

3. To evaluate the applicability of biomagnification for increasing the analytical sensitivity for the measurement of organic compounds in raw public drinking water supplies; and

4. To assess the feasibility of using a fish physiograph unit to detect elevated levels of organic compounds in raw drinking water supplies, with particular emphasis on the development of such equipment for use as an abatement tool to continuously monitor surface water quality.

2.3 STUDY APPROACH

In this research study, IEC assessed the feasibility of two biological methods to monitor organics levels in drinking water supplies: one with long-term monitoring and abatement applications (bioaccumulation) and the other to serve as an alarm indicator of sudden, increases in organics levels (fish physiograph). Fish were used as test organisms as previous studies had shown them to be practical and suitable biological indicators (1, 2).

Bioaccumulation permits the measurement of the bioconcentration of organic compounds by fish. In this case, compounds are taken up by the fish and concentrated in their tissues at levels higher than present in water. Chemical analysis of organics accumulated in fish permits an assessment of the degree of water contamination by organics as well as the biological affinity of the organics and their potential human health risks. Furthermore, this method enables measurement of organic compounds below their detection levels in water and identification of infrequent discharges or spills of organics.

The fish physiograph method permits the measurement of respiratory changes in fish as a possible response to organic contamination of water. Successful demonstration of this biomonitoring technique may enable the use of the fish physiograph as an early warning system to signal the occurrence of elevated concentrations of organic compounds and subsequently provide time for remedial action such as additional treatment of the raw water supply.

In order to facilitate review of this report, IEC has annexed relevant references at the end of each section. In addition, the two main sections 3.0 on bioaccumulation and 4.0 on fish physiography contain extensive literature reviews at the outset of the sections. These reviews will assist readers who may not be fully conversant with the subject matter.

2.4 STUDY AREA

The Grand River Basin was selected as the study area (Figures 2.1 and 2.2). This 6,671 km^2 drainage basin flows into Lake Erie and forms the largest watershed in Southern Ontario. Six major urban and over twenty rural communities are located in the drainage basin, which has an estimated population of 600,000.

Prior to 1976 many industries discharged effluents directly into the Grand River and its tributaries. Most of these effluents are now being diverted to sewage treatment plants.

Ground water has been used for drinking water by all communities except the City of Brantford and the Town of Cayuga. Increased demands have recently resulted in a partial switchover to surface water as a drinking water source in the region of Kitchener-Waterloo.

Brantford was selected as the experimental site for both the fish physiograph and bioaccumulation studies since the city is dependent on the Grand River as a drinking water source and municipal and industrial outfalls occur upstream of the Brantford water treatment plant intake. The control site for the bioaccumulation study was located on Galt Creek, a small tributary which drains an undeveloped portion of the watershed and joins the Grand River at Galt, 25 km upstream of Brantford.

The two sites were chosen on the basis of the following selection criteria:

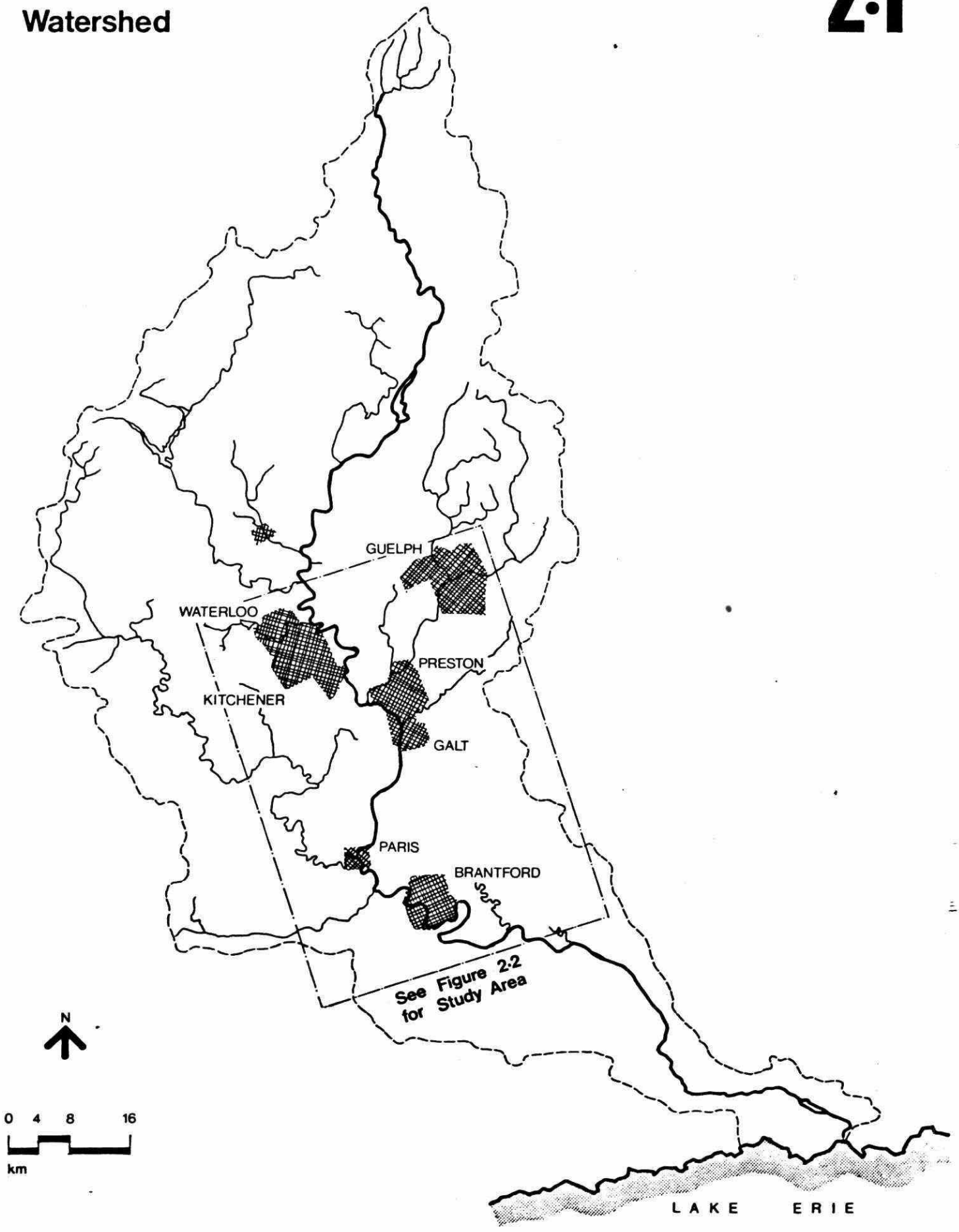
1. The presence of a municipal raw water intake at the downstream experimental station;

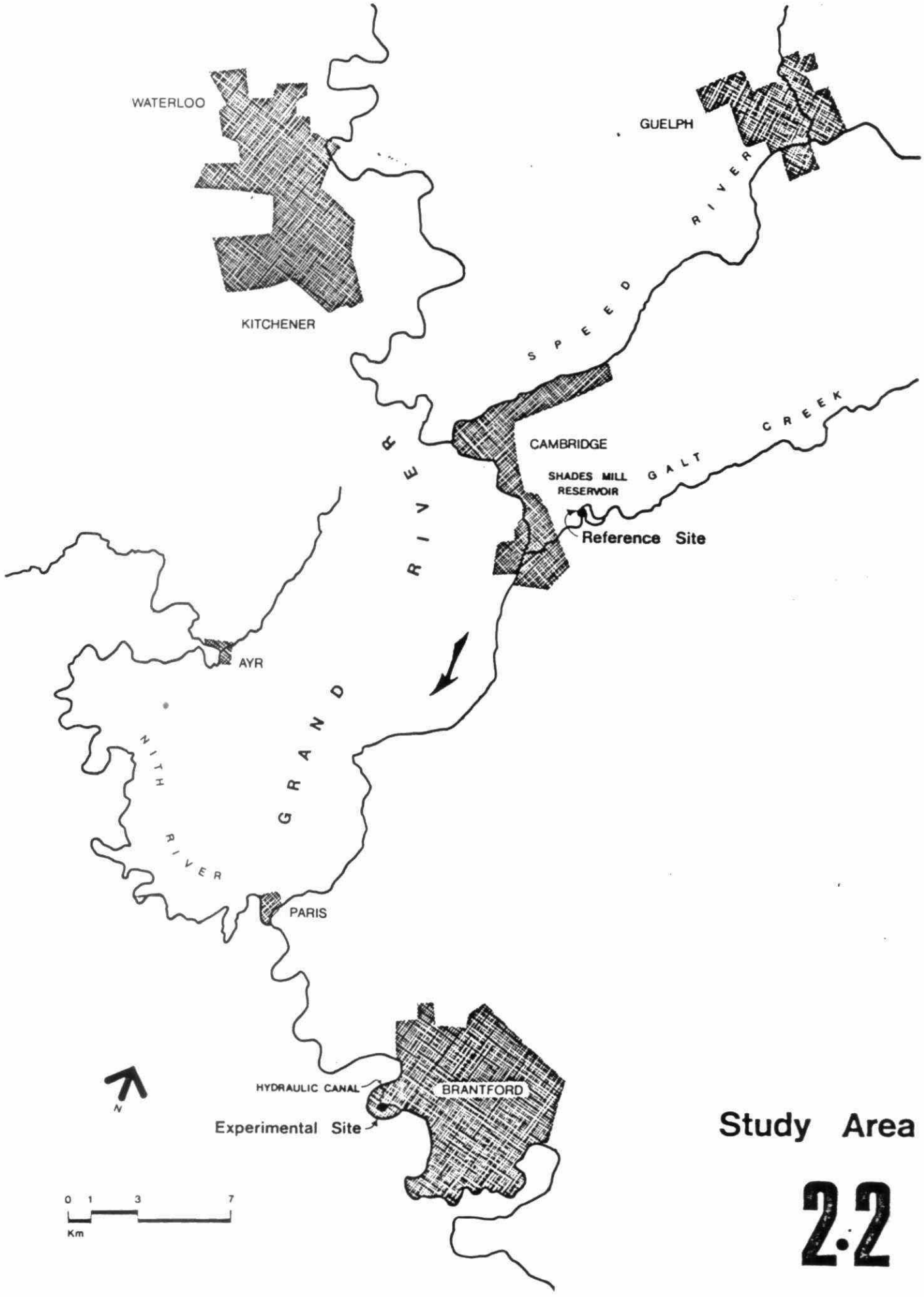
2. The presence of municipal and/or industrial effluent discharges upstream of the downstream experimental station;

3. The least number of point source discharges above the upstream control station;

Grand River Watershed

2.1





Study Area

2.2

4. Permanent flow throughout the entire year at both study sites;

5. Water typical of the majority of potable water supplies within the province having a minimal natural organic content;

6. Suitable sites for the installation of the research laboratory and intake lines;

7. Availability of appropriate utilities; and

8. Approval by the municipality concerned.

Final selection of the sites was made by IEC in conjunction with MOE. Authorization to establish a facility in the Water Treatment Plant at Brantford was given by Mr. Bob Wilson, General Manager Brantford Public Utilities Commission while Mr. Ilmar Kao, Assistant General Manager, Grand River Conservation Authority gave approval to assemble a monitoring facility in the dam building of Shades Mill Reservoir in Galt.

2.5 REFERENCES

1. Phillips, D.J.H. 1978. Use of biological indicator organisms to quantitate organochlorine pollutants in aquatic environments: A review. Environ. Pollut. 16:167.

2. Cairns, J., Jr. and D. Gruber. 1980. A comparison of methods and instrumentation of biological early warning systems. Water Resources Bull. 16:261.

3.0 BIOACCUMULATION

3.1 LITERATURE REVIEW OF BIOACCUMULATION OF ORGANICS

3.1.1 Organics in Water

Production of synthetic organic chemicals has grown exponentially (1). More than four million chemicals are known today and 1,000 new chemicals are introduced each year (2). Of these, over 10,000 chemicals are produced in excess of 500 kg annually in the world (3). United States production of halogenated organic compounds exceeds 5 million tons (4) and world production of chlorinated aliphatic hydrocarbons in 1971 was 3 million tons (5).

Consequently, surface waters treated for drinking may contain many organic compounds in addition to those of natural occurrence. Trace amounts of these organic compounds may enter water either intentionally or accidentally. As a result, there is a potential for human intake of these organics.

Recent developments in analytical methods such as the coupling of gas chromatography and mass spectrometry have enabled the identification of large numbers of synthetic chemicals in water. A 1975 study identified more than 400 different organics in raw water and 325 in finished drinking water (6). Other studies have found 187 organic compounds in samples of U.S. tap water (7) and about 300 organics in drinking water of which many were known or suspected carcinogens, mutagens or teratogens (8). Recently, a list of 7,000 literature entries on organics in different water types identified 1,300 different compounds (9). Many of these compounds are known to cause public health problems and are therefore registered with the EPA List of Priority Pollutants (10) and the National Academy of Sciences List of Carcinogens (11).

In spite of the large number of compounds already identified in water it is now recognized that present analytical methods are suited to identifying volatile organics which comprise about 10% of total organic carbon in water (12, 13, 14). The polar and larger molecular weight molecules such as nitrosamines and chlorinated non-volatiles are generally not measured. These organics in drinking water may also pose a health hazard, as there is no evidence to indicate that these unmeasured compounds are harmless.

There is evidence to suggest that health effects of trace organics only appear following a long latency period. For example, vinyl chloride and phthalate esters were shown to have carcinogenic and teratogenic effects after 30 to 40 years of commercial use (15). Several researchers have suggested that the majority of human cancers are induced by environmental agents and correlations between the consumption of polluted water and the incidence of cancer have been demonstrated (16, 17, 18, 19). The high lipophilicity, stability and wide usage of many organic chemicals underscores the need for further development of techniques to monitor low levels of organics in drinking water.

3.1.2 Bioaccumulation in Humans

Research of the effects of organics in water on human health deal primarily with chronic exposure. Man is intermittently exposed to low levels of organics through direct intake of water. The consumption of aquatic organisms which may have significant concentrations of organics due to bioconcentration and biomagnification processes represents an additional pathway for human exposure. Table 3.1 presents available data on 15 organic priority pollutants measured in human adipose tissue. Some of the data such as dieldrin showed significant levels above those found in food (24).

However, food consumption is a more important pathway contributing to organic residue levels than water intake or other exposure sources (21, 31, 32, 33, 34, 35, 36, 37). A million litres of water would have to be

TABLE 3.1: LEVELS OF ORGANIC CONTAMINANTS IN HUMAN ADIPOSE TISSUE

Compound	Concentration (ppm) Mean	Range	Location	Reference
α BHC	0.004	0.001-0.036	Canada	(20)
β BHC	0.054	0.001-1.790	"	(20)
Lindane (BHC)	0.007	0.001-0.136	"	(20)
"	0.015	<0.01-0.17	"	(21)
Oxy-chlordane	0.055	0.003-0.336	"	(20)
Trans-nonachlor	0.065	0.010-0.367	"	(20)
DDT and metabolites				
(1969-70)	7.59	1.32-29.6	"	(22)
(1971-72)	5.67	0.49-15.5	"	(22)
(1973-74)	3.69	0.46-13.4	"	(22)
(1969)	4.54	0.18-18.71	"	(21)
(1972)	2.57	-	"	(20)
Dieldrin (HEOD)	0.069	0.001-0.353	"	(20)
"	0.122	0.02-0.46	"	(21)
"	0.13	ND-1.30	"	(22)
"	0.16	-	"	(23)
"	0.18	-	U.S.A.	(24)
Endrin	0.02-0.3	-	"	(24)
"	0.02	-	England	(25)
HCB	0.11	ND-0.50	Canada	(22)
"	0.062	0.001-0.520	"	(20)
"	0.09	0.05-0.13	"	(26)
Heptachlor epoxide	0.043	0.003-0.477	"	(20)
"	0.040	<0.01-0.20	"	(21)
PCB's	0.907	0.106-6.603	"	(20)
"	0.70	0.41-1.16	"	(26)
"	2.0	ND-18	"	(22)
PCP	0.023	0.010-0.080	U.S.A.	(27)
"	0.14	ND-0.57	Japan	(28)
"	0.025	0.005-0.052	U.S.A.	(29)
Chloroform	0.160	0.020-0.460	"	(30)
DBP	0.63	0.52-0.79	Canada	(26)
DEHP	0.81	0.64-1.15	"	(26)

consumed containing organochlorine pesticides and PCB's to equal the exposure resulting from a single meal of Great Lakes trout or salmon (15). Furthermore, contributions of chloroform from toothpaste could be as important as water intake to total daily body intake (38). Assuming an intake of two litres of water per day and a chloroform concentration of 300 ug/L, contributions from drinking water would be 15 times lower than the acceptable daily intake and therefore 1,500 times below the lowest exposure level shown to cause health effects.

Organochlorine residues were found in Atlantic coast fish with higher concentrations occurring in fish species with greater fat content (39). Dieldrin concentrations of 1 ppb or higher were analyzed in all meat, fish and poultry samples and in 97% of dairy products sampled (24). Meat, fish and poultry contained organic residues more frequently than any other food products (40).

Food is the primary source of organics. Lower organics concentrations have been reported in meat abstainers (41). Similar pesticide levels found in Americans from three widely separated states were due to the uniformity of food eaten (34). Ingestion by Japanese of fish containing 0.40 - 0.90 ppm PCB's resulted in elevated PCB blood levels compared to the same population fed rice or pork and rice meal (42).

General use of DDT was banned between 1970 to 1973 resulting in a decline of residue levels in Canadians as shown in Table 3.1 (22, 43). The increase during this period in the proportion of pp DDE, a stable and slowly excreted metabolite of dietary origin, illustrates the significant contribution of diet on total intake.

Other sources such as dust, air, tobacco and cosmetics must also be considered when assessing total human intake of organics. However, organic contribution by these pathways is generally lower. For example, assuming exposure levels of dieldrin in the air to be as high as those recorded at times in agricultural areas (20 ng/m^3), then the total contribution to daily intake would be 2.6 times lower than diet contribution (24).

Organic residues in stillborns demonstrate accumulation during fetal life (44). PCB's, dieldrin and DDT are transferred during lactation and result in a net loss of organic residues from the mother to the infant based on daily intake from food whereas placental transfer was not as great as lactation transfer (37, 45).

Various factors influence the bioaccumulation of organic compounds in humans. The lipophilicity of the compound is established as a factor of primary importance. The chemical structure of the organic compound may also affect the body's ability to metabolize and eliminate it. Researchers have demonstrated that PCB's which have 1 or 2 chlorine atoms at the ortho position of the biphenyl bridge are more readily retained in the human body (42). However, it has been shown that quantitative differences in storage of compounds does not parallel their fat solubility (41). Various influencing factors have been proposed to explain this observation, including intensity and duration of exposure, sex, race, nutritional status and living and working conditions.

The direct relationship between DDT dosage and level of accumulation has been clearly demonstrated (35). Residents of Southern Ontario living in areas of extensive previous use of DDT had slightly higher pp' DDT residue levels than those residents from the rest of the province (22). Higher blood serum levels of PCP in residents of Hawaii compared to people living in Florida was explained by differences in PCP usage in the two locations (27). In Mississippi, human milk samples from women in an area of high pesticide usage were significantly higher than those living in a low pesticide usage area (46). Regional difference in residue levels of Canadians have been documented but have not been explained (20).

Researchers have found that males have higher mean tissue residue values than females (20, 21, 44). However, these differences were not great and significant differences between male and female Canadians have only been reported for trans-nonachlor probably due to degree of exposure.

Generally, human residue concentrations increase with age. In two Canadian studies, 7 of 13 compounds identified in adipose tissue were found to occur

at higher concentrations in older subjects (20, 21). However, significant differences between age groups were found only for pp' - DDE, dieldrin and heptachor epoxide. A U.S. study indicated that DDT and its analogs were racially stratified with Caucasians having lower residues than Blacks of the same age (43).

Interaction between organics has been reported to affect bioaccumulation. The presence of DDT in human tissue has been observed to inhibit dieldrin storage. This finding was explained on the basis of biochemical interaction where DDT impaired the detoxification of dieldrin (44). Different residue levels in tissues and organs have been explained by organ function (eg. detoxification in liver and bile) and different lipid types (eg. low organic levels in brain tissue due to low neutral lipid content).

In spite of the extensive literature on organics levels in humans and organic sources and organic occurrence, little is known of organic health effects. No diseases have been linked with organic residues, eg. pesticide formulators with body burdens of more than 200 ppm DDT have shown no visible effects (41).

As discussed, health effects of trace organics appear generally following a latency period and may be exhibited in various ways. For example, reproductive failure was reported in commercially raised mink fed PCB-contaminated coho salmon from the Great Lakes (15). Moreover, a connection has been suggested between organic contamination of Great Lakes salmon and the high incidence of tumors in their flesh (47, 148). These examples of health effects in other organisms associated with ingestion of food and/or water from the Great Lakes suggest the need for surveillance programs to monitor low level contamination of food and water in the Great Lakes Region.

3.1.3 Bioaccumulation in Fish

The bioaccumulation of organics in fish is well documented (48, 49, 50). Tissue levels in the parts per million range of DDT, HCB and PCB's may be 10^6 times greater than water concentrations which are in the parts per trillion range (51, 52, 53). Therefore, organic bioaccumulation in fish enables monitoring of trace organics in water (54) and the same organics may bioaccumulate in humans and possibly pose health hazards. For example, the U.S. National Monitoring Program analyzes organic levels in fish to establish trends of increased accumulation and to assess the potential for human exposure.

Several studies have used fish to monitor organics in water. For example, rainbow trout were used to accumulate organics from Rhine River water. The results of the tissue analyses suggested a potential human health hazard of this drinking water source (55). In another study, the impact of aldrin spraying was assessed by monitoring fish residues and comparing them to human food guidelines (56). By monitoring fish residue levels, other studies have demonstrated correlations between tissue residues and runoff dilution; documented PCB contamination in an area of recreational and commercial fishery importance; and followed DDT decline in the environment since its ban (57, 58, 59). Fish have also been used to monitor the disappearance of trifluralin in a river following installation of an activated carbon treatment process (60).

Bioaccumulation can occur via two pathways, through the food chain (ie. biomagnification) and through direct contact with water (ie. bioconcentration). There is controversy over the relative importance of these pathways. Laboratory exposures of fish to organic concentrations to which their food had been exposed showed proportionally higher organic contributions from water than from the food (61, 62, 63, 64, 65).

In contrast, laboratory studies exposing fish to organic levels in water and food similar to those found in the Great Lakes showed that dietary sources were the most important (49, 66).

It has been suggested that DDT and a number of other organics with low elimination rates have accumulation potential in the food chain (65). However, for most organics, biomagnification has been shown to be a less significant pathway. Concentration factors in fathead minnows exposed to laboratory water were similar to bioconcentration factors (BCF) found for small fish in rivers (52, 67, 68, 69, 70). However, uptake via the diet may be more significant than uptake from water low in organics such as Great Lakes water. For example, PCB uptake by Lake Michigan lake trout was shown to occur almost completely via the sediment based food chain (71).

The intensity and duration of exposure influences the types and levels of organics accumulated by fish. DDT residues declined in young salmon after the forest spraying season (72). Similarly, different residue levels in fish from the Grand River corresponded to seasonal availability of domestic and agricultural pesticides (73). Fish residue concentrations of trifluralin discharged into a river varied inversely with river flow (60). Furthermore, increased PCB levels in lake fish sampled in the spring were attributed to remobilization due to sediment erosion in feeder streams (74).

Continuous laboratory exposure of fish to controlled concentrations of 31 "priority" pollutants including various halogenated benzenes, phenols, methanes, ethanes, ethers and alkenes as well as several PAH's and phthalate esters demonstrated their propensity to accumulate and persist in fish (50, 53). Most accumulated rapidly and reached an equilibrium concentration within 3 to 10 days of exposure. The half-life of elimination was equally rapid and usually less than 7 days. A few organochlorine compounds took longer to reach a steady-state concentration eg. 140 days for dieldrin, and to be eliminated eg. 160 days for DDT (66, 75).

Many studies of bioaccumulation have utilized organochlorine pesticides and the industrial chemicals HCB and PCB's (74, 76, 77, 78, 79, 80, 81). These organics have considerable environmental significance due to their high lipophilicity and persistence because of the stability of the chlorinated benzene ring (75, 82, 83). The early studies of this group dealt with the bioaccumulation of DDT and its analogs followed by studies of mirex,

endrin, aldrin and dieldrin (77, 84, 85). A laboratory model ecosystem approach was used to compare the fate of chlorobiphenyls with DDE (86).

Bioaccumulation has also been used as a standard determinant of the environmental and health hazard potential of many other organics (87). Bioconcentration potential has been determined for a vast number of organic compounds representing at least 13 chemical classes including: phenols, chlorinated benzenes, ethanes and ethylenes, phtalates, ethers, simple benzene derivatives, the plasticizer di-n-octylphthalate (DOP), unsubstituted aromatic hydrocarbons, and chlorophenols (51, 53, 88, 89, 90, 91). These studies have revealed that structural properties determine the bioaccumulation of an organic compound based on water solubility and degradability.

The residue forming potential of these compounds is expressed as a bioconcentration factor or BCF and calculated as the measured concentration of a chemical in fish tissue at steady-state divided by the measured concentration of the chemical in water during the exposure. Laboratory determinations of bioconcentration factors have revealed that aromatic hydrocarbons are generally bioconcentrated to a greater degree (BCF of 57 to 3400) than aliphatic organics (BCF of 2 to 387) (53). Also, the accumulation of specific aromatic hydrocarbons increases with the number of fused rings in the molecule. For example, anthracene accumulates more than naphthalene which accumulates more than benzene (90). In addition, accumulation increases in proportion to the extent of ring substitution such that alkyl-substituted aromatics are accumulated more than unsubstituted structures. This general principle also applies to chlorobenzenes, chlorobiphenyls and chlorophenols (53, 81, 91).

The form of the ring substitute is important in determining the biodegradability of simple benzene derivatives (88). For example, metabolism of the parent compound in Gambusia was greatest when COOH was the substituent followed by NH_2 and NO_2 with Cl being the least biodegradable. Moreover, accumulation potential of tetrachlorobiphenyls and trichlorophenols was reported to be dependent on the position of the substituent on the benzene ring (91, 92).

Other factors in addition to exposure and chemical properties may influence bioaccumulation. These factors include fish species characteristics such as age, lipid content, sex and condition and environmental factors such as temperature and season of the year.

Differences in bioaccumulation have been demonstrated between species for the same compound. Elimination rates for DDT although similar for Salmo salar and Salmo gairdneri were much faster in Salvelinus fontinalis (72, 93, 94). Higher accumulation of Aroclor 1254 occurred in fathead minnows and green sunfish than in rainbow trout (53). Differences in temperature optimum for enzyme detoxification may account for the differences observed between the warm water and the cold water species (89).

Different species body lipid content may also be an important determinant as strong correlations occurred between lipid solubilities of organics and bioconcentration potential in fish (52, 76, 95, 96, 97, 98). Accumulation potential differences within and between species may be reduced by basing organics content on lipid weight instead of wet weight of the fish (99, 100, 101). However, this difference presupposes water partitioning is more important than dietary sources. It has been recommended that organic levels in fish be determined on both a lipid content and wet weight basis to avoid difficulties in interpretation (102). In a seven month study of organochlorine accumulation in herring from the North Sea, it was determined that although an increase in residues was shown based on wet weight, this increase could not be correlated with an increase in ambient organic levels and the increase was related instead to an increase in lipid content with age (103).

Different organic residue concentrations may also be due to age, migration and metabolic rate. Differentiation of effects due to age, weight, size and other covariates such as season are difficult. DDT residues were found to be proportional to fish size although sex and condition affected the correlations (99). In a study using data compiled for the U.S. National Monitoring Program, no conclusions regarding the effect of fish size could

be made due to the small numbers of fish sampled (104). PCB levels in trout from Cayuga Lake were significantly correlated to age, length and weight, although correlations with age provided the best fit (105). Increased organochlorine concentrations based on wet weight were reported in older fish; however, different concentrations due to age were not found on a lipid basis (100, 106). Nevertheless, other factors in addition to lipid content affected age-based differences in organics levels (107).

Uptake rates may be different in fish of different age or size. Small fish were found to respond more quickly to trifluralin discharges to a river suggesting more rapid uptake (60). Faster uptake of DDT by smaller fish of the same species has been reported (108). Furthermore, DDT uptake in fish of different sizes has been correlated with oxygen consumption, ie. metabolic rate (109). These studies suggest that higher organic uptake is due to the higher metabolic rate of the smaller fish.

Seasonal temperature changes affect the availability of organics and the lipid content of fish which in turn affects bioaccumulation potential. Several authors have observed seasonal profiles of accumulation due to changes in availability (72, 73, 101). One study reported that seasonal peaks were correlated more closely with spring runoff than with application time (110). Increased PCB uptake by roach has been shown to occur during the spawning period and was likely due to increased activity (74). Organochlorine concentrations in female salmon were reported to be dependent on their sexual condition (107). It is reasoned that seasonal elevations in water temperature increase the solubility of many organics and their availability to fish (111).

A number of studies have shown that interactions between organics affect bioaccumulation. For example, the presence of dieldrin in fish tissue has been shown to enhance DDT storage in rainbow trout, while DDT decreased dieldrin accumulation (75). In a similar study, methoxychlor decreased storage of DDT and dieldrin, whereas DDT decreased dieldrin accumulation (112). Furthermore, dieldrin increased methoxychlor storage while it was

inhibited by DDT. These effects may be due to the induction of metabolizing enzymes in fish liver such as microsomal oxidase (112). In contrast, no interaction was seen beween PCB and dieldrin in white suckers (113).

Because of the many factors affecting bioaccumulation potential of organics in water, rigorous sampling and interpretive procedures are needed to permit the monitoring of organics in water using indigenous fish. An alternate monitoring approach consists of using hatchery fish of uniform age, size and condition. If control of biological influences are taken into account, fish can be used as effective monitors of a number of organic compounds to identify unknown or infrequent discharges. The duration of exposure can determine the class of compound accumulated according to its reactivity and persistance. As such, hatchery fish may be used to identify specific sources of pollution because residue levels provide a direct measure of the biological availability of pollutants and an estimate of the intensity and duration of exposure. Finally, a quantification of organic levels in water is possible using fish to accumulate compounds present at concentrations too low to be measured by either routine or available analytical technology. This monitoring approach is discussed in subsections 3.2 through 3.5 of this report.

3.2 RATIONALE FOR EXPERIMENTAL APPROACH

Recent advances in analytical technology, in particular the coupling of gas chromatography and mass spectrometry and the use of computer-based spectral data banks, have enabled the identification of 1,300 organics in water (9). While a 1970 U.S. survey identified only 66 of 496 organic compounds suspected in water, a 1976 compilation listed 1,259 organics (114, 115). Moreover, about 400 organic compounds have been identified in finished drinking water (116, 117, 118).

Research is currently addressing the distribution and fate of organic compounds in natural waters to determine their possible health significance. Of the organic compounds investigated to date a few, notably DDT, HCB, and PCB's, pose human health risk at low concentrations due to their accumulation potential. Analyses of water samples collected during routine sampling programs rarely detect these compounds and concentration techniques must be used to measure the organics at detectable levels.

Fish have been demonstrated to accumulate organics in tissue at levels higher than those in water. In some cases the accumulation is 10^6 fold (from ppt in water to ppm in the tissues) (50,51, 52, 53). DDT, BHC and dieldrin are known to accumulate in fish and human tissues and therefore can be used as indicators and monitors of organic contamination (41, 54, 75). Fish not only accumulate organics to detectable levels but also screen them according to their biological activity and likelihood of becoming cumulative poisons. A recent European study of a public water supply on the Rhine River showed rainbow trout accumulated substantial amounts of the five persistent organochlorine compounds during an 18 month exposure period (55).

Bioaccumulation is broadly defined as the net accumulation of chemical residues in tissue, and it occurs through two pathways: bioconcentration, or direct partitioning from water, and biomagnification or accumulation from dietary sources. Field and laboratory studies indicate that bioconcentra-

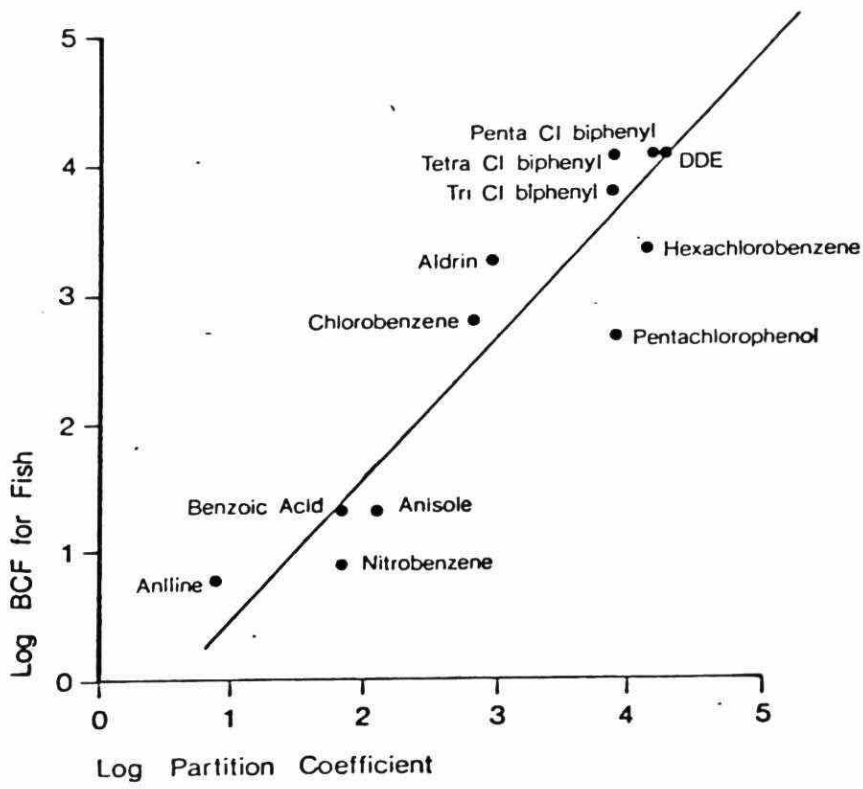
tion is the major pathway of bioaccumulation (64, 65, 67, 119). Biomagnification appears to be important to body burden accumulation for those compounds that are highly persistent in fish such as DDT or when levels in the water are low (50, 65, 71, 120).

Major biological factors which influence bioaccumulation include: the age and size of the fish, health, lipid content and metabolic rate of activity (52, 56, 58, 60, 74, 76, 79, 109, 121). Since biological membranes are composed of lipids, lipid soluble and neutral molecules penetrate fish most readily. Researchers have demonstrated a linear log relationship between the solvent/water partition coefficients of a number of organic chemicals and their bioconcentration potential (Figure 3.1) (52, 97, 122). Once an organic compound penetrates a fish, properties such as polarity and reactivity determine its fate. The end result is not always predictable. Sometimes a persistent compound is formed from the parent compound as in the case of heptachlor and its metabolite heptachlor epoxide (84). The parent compound may be activated to a more toxic form as in the oxidation of parathion to paraoxon or a relatively innocuous compound may be converted to a compound of greater bioconcentration potential as in the case of benzoic acid and its metabolite hippuric acid (88,123).

A number of general comments can be made concerning the bioconcentration process. Laboratory studies have shown bioconcentration to occur rapidly in most cases. Fish continuously exposed to a constant organic concentration usually reach a steady state body burden within ten days and maintain the equilibrium unless there is a change in the exposure situation (53). Transfer of contaminated fish to clean water results in rapid elimination of most compounds with resultant biological half-lives of less than seven days. However, organochlorine compounds that are highly lipophilic and nonreactive take longer to reach equilibrium and to be cleared from the body. These bioconcentration procedures are used to estimate the bioconcentration factor of a compound, expressed as BCF, which is the measured concentration of the chemical in fish tissue at equilibrium divided by the measured concentration of the chemical in water during the exposure. BCF's for various organics are presented in Table 3.2. Evidence indicates that the time to reach equilibrium, biological half-life and BCF are independent of exposure concentration (50, 52, 53).

Log BCF for Fish vs Log Octanol/Water Partition Coefficient

52,78,86,88



Equation (52)

Log BCF : 0.85 Log P - 0.70

TABLE 3.2: BIOCONCENTRATION AND PERSISTANCE OF 35 ORGANIC PRIORITY POLLUTANTS (50, 52, 53)

Organic Compound	Concentration (ug/L)	Days to Equilibrium	Bioconcentration Factor (X)	Half-life (days)
DDT	0.003 6.5	>120	8,500 29,400	160
Dieldrin	2.0	140	10,000	40
Heptachlor	2.0 3.1	-	>10,000 9,500	<28
Hexachlorobenzene	5.0 2.6	14	>20,000 18,500	>60
Aroclor 1254	1.0 4.3	28	37,000 100,000	42
Dioxin (TCDD)	0.24	-	12,000	-
Tetrachlorobiphenyl	14.0	>48	12,400	30
Toxaphene	0.5	>140	16,000	>50
Acenaphthene	8.94 ± 2.13	>3<10	387	<1
Acrolein	13.1 ± 2.64	>3<10	344	>7
Acrylonitrile	9.94 ± 1.16	>28	48	>4<7
1,2-dichlorobenzene	7.89 ± 1.20	>3<10	89	<1
1,3-dichlorobenzene	107 ± 10.9	>3<10	66	<1
1,4-dichlorobenzene	10.1 ± 0.75	>3<10	60	<1
1,2,4-trichlorobenzene	2.87 ± 1.08 1.6	>3<10	182 2800	>1<3
1,2,3,5-trichlorobenzene	7.72 ± 0.59	>28	1,800	>2<4
Pentachlorobenzene	5.15 ± 2.20	>3<10	3,400	>7
Carbon tetrachloride	52.3 ± 12.7	>3<10	30	<1
Chloroform	110 ± 6.6	>3<10	6	<1
1,2-dichloroethane	95.6 ± 11.1	>3<10	2	>1<2
1,1,1-trichloroethane	73.4 ± 14.3	>3<10	9	<1

TABLE 3.2: BIOCONCENTRATION AND PERSISTANCE OF 35 ORGANIC PRIORITY POLLUTANTS

Organic Compound	Concentration (ug/L)	Days to Equilibrium	Bioconcentration Factor (X)	Half-life (days)
1,1,2,2-tetrachloroethane	9.62 ± 1.09	>3<10	8	<1
Pentachloroethane	7.93 ± 0.49	>3<10	67	<1
Hexachloroethane	6.17 ± 1.95	>3<10	139	<1
Bis(2-chloroethyl)ether	9.91 ± 0.43	>3<10	11	>4<7
1,1,2-trichloroethylene	8.23 ± 0.42	>3<10	17	<1
Tetrachloroethylene	3.43 ± 1.53	>3<10	49	<1
Isophorone	92.4 ± 10.5	>3<10	7	1
N-nitrosodiphenylamine	9.21 ± 0.98	>28	217	<1
2-chlorophenol	9.18 ± 2.02	>3<10	214	<1
2,4-dimethylphenol	10.2 ± 0.76	>3<10	150	1
Dimethylphthalate	8.74 ± 1.90	>3<10	57	>1<2
Diethylpthalate	9.42 ± 2.89	>3<10	117	>1<2
Butylbenzylphthalate	9.73 ± 1.75	>3<10	663	>1<2
Di-2-ethylhexyl phthalate	5.82 ± 0.90	>3<10	114	3

In IEC's research fish were used as accumulators of organics in the public water supply at Brantford to assess and compare data obtained using a chemical concentrating material. State-of-the-art techniques were utilized. Fish and chemical absorbent material were periodically analyzed for all classes of organic contaminants. Results at two sites, one on the developed and the other on the undeveloped portion of the Grand River watershed were compared to differentiate organics of natural or man-made origin.

3.3 MATERIALS AND METHODS

3.3.1 Experimental Design

The experimental design involved simultaneous exposure of rainbow trout fry to untreated municipal water at the upstream reference and the downstream experimental site for a 12 week period. Fish were collected for organic residue analysis at both sites for weeks 0, 1, 2, 4, 8 and 12 of the exposure. During the experiment, accumulator resin cartridges with one to two week lifespan were installed at each site to concentrate organics from the water for subsequent organic chemical analysis to compare organic levels with the organic levels and compounds accumulated by the trout. Comparison of results for the upstream rural, undeveloped site with those at the downstream site located on a developed portion of the watershed allowed differentiation of natural organics from those contributed from anthropogenic sources.

3.3.2 Site Selection

The experimental sites for this study were located on the Grand River watershed, a large, multiple use river basin in Southern Ontario (Figures 2.1 and 2.2). The surface water at both sites was similar in basic water quality characteristics (Table 3.3). However, the types and levels of organic compounds at each of these locations were expected to differ due to differences in land use of the drainage areas.

The upstream reference site was situated at Shades Mill Reservoir, a small man-made lake fed by Galt Creek, a small tributary of the Grand River with steady flow throughout the year. Local municipalities use the reservoir for ground-water recharge of public water supplies. Galt Creek drains rural, agricultural and undeveloped wooded areas and has no known pollution point-sources. All available water quality data indicated that the organic pollutant levels in the water of this area were low and usually below detection (124).

TABLE 3.3: BASIC WATER QUALITY PARAMETERS[1]

Parameter	Brantford, Grand R.	Shades Mill, Galt Creek
Temperature (°C)		
January	0.0	0.0
February	0.0	0.6
March	0.5	--
April	5.5	0.0 - 14.5
May	16.0	13.0 - 22.0
June	22.0	13.8 - 26.0
July	23.0	18.0 - 28.7
August	23.5	15.0 - 24.5
September	17.0	10.8 - 20.5
October	11.0	2.5 - 15.8
November	2.0 - 9.0	0.0 - 7.8
December	0.0 - 5.0	0.0 - 1.8
pH	7.7 - 8.6	7.8 - 8.4
Ca (mg/L)	73	55
Mg (mg/L)	20	22
Total Alkalinity (mg/L $CaCO_3$)	173	187
Conductivity (umhos/cm))	300 - 900	300 - 520
Suspended Solids (mg/L)	3 - 1490	1 - 18
Dissolved Oxygen (mg/L)	high levels throughout the year, both sites 7.6 - 12.0	

[1]Mostly mean values, ranges included when available.

The Brantford Water Treatment Plant drawing from the Grand River was selected as the downstream experimental site. The location was chosen as it is situated downstream of diffuse and point source discharges arising from four major urban industrialized areas (124). Also, taste and odour complaints have been periodically registered in the Brantford potable water supply.

Criteria used to select the two sites were discussed in Section 2.

3.3.3 Laboratory Facilities

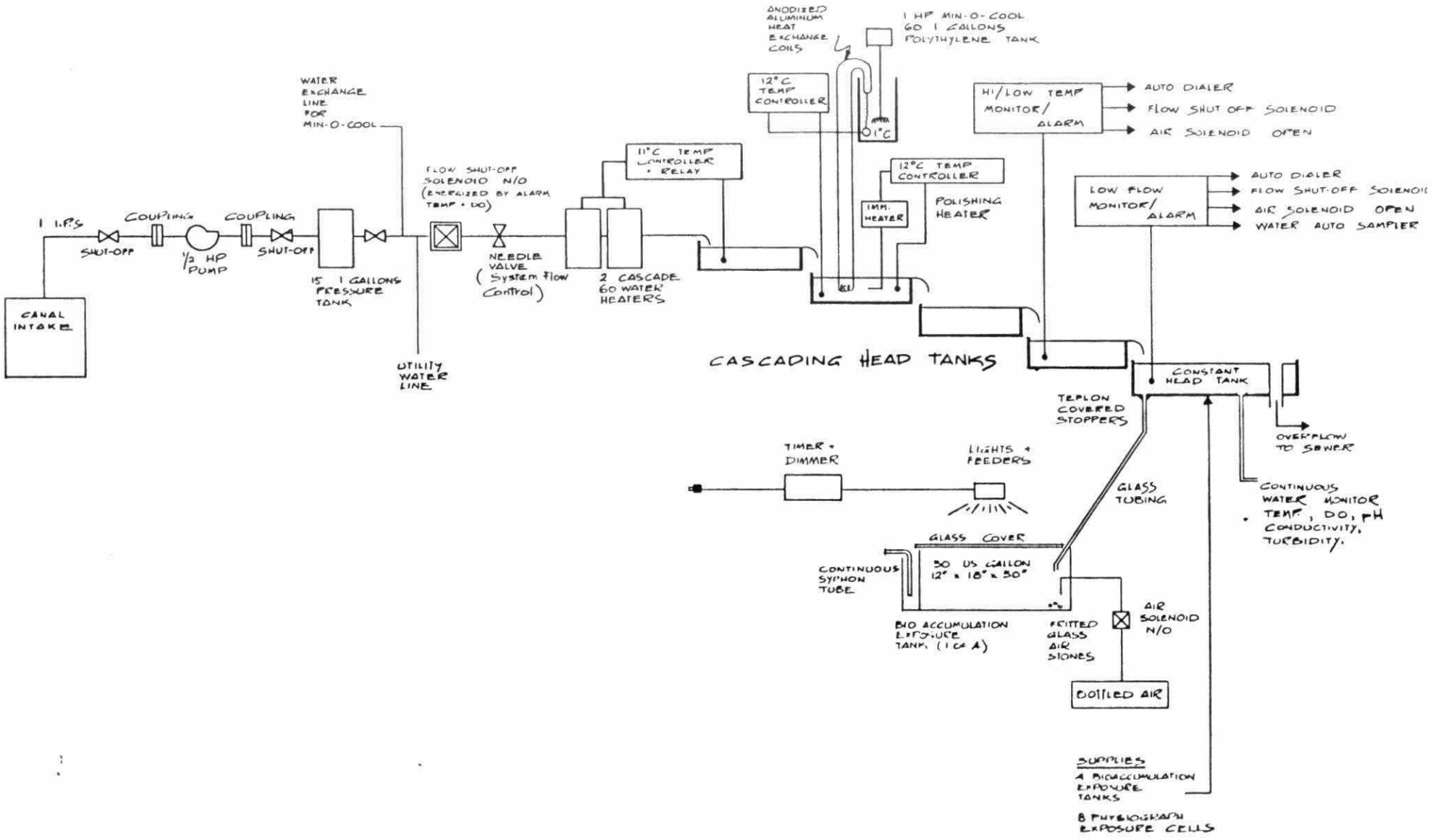
Research laboratories were constructed at both sites for the fish exposure facilities (Figure 3.2). Each laboratory was capable of providing continuous flow (10 L/min) of temperature controlled and aerated water drawn from the water supplies without retention or dilution (Figures 3.3 and 3.4). To minimize leaching or dissolution of substances into the water or sorption of organics from the water, all materials which contacted the exposure water were made of degreased iron, glass or perfluorocarbon plastics. Loss of trace amounts of volatile organic compounds through aeration of the exposure water was minimized by cascading the water between head-tanks instead of using bubblers. This also alleviated any possibility of supersaturation of the water with gases following heating.

Water monitors provided continuous measurement of turbidity, conductivity, dissolved oxygen, pH and temperature at the two sites. Fail-safes protected against loss of fish through power failure, variation in water temperature or changes in flow. Activation of the fail-safes triggered flow shut-off valves and simultaneously activated oxygen flow from compressed gas cylinders to the exposure tanks to maintain the level of dissolved oxygen. Each laboratory was equipped with automatic telephone dialers which activated during alarm conditions and alerted the on-site biologist so that corrective measures could be taken.

Lab Flow Plan

BRANTFORD

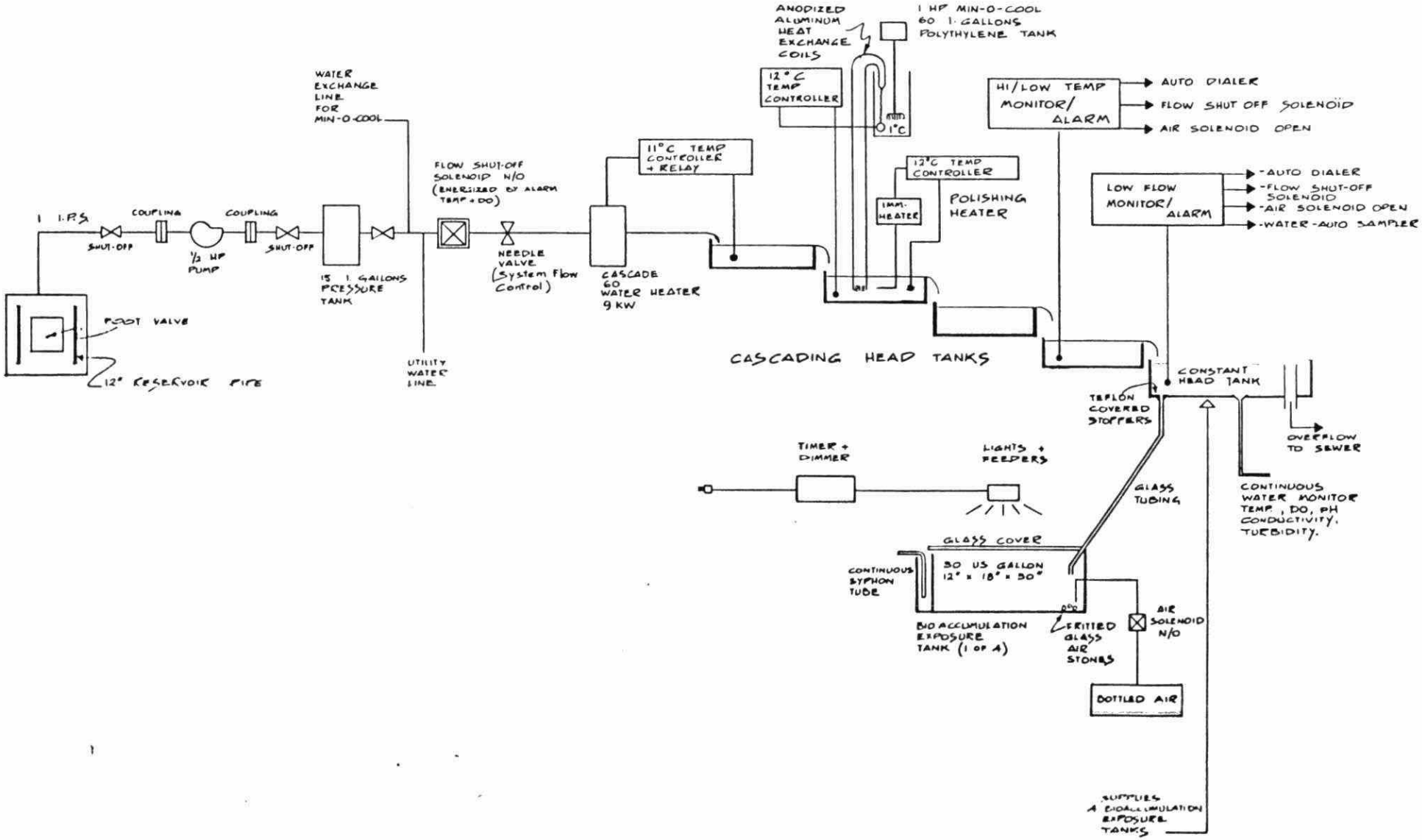
3.3



Lab Flow Plan

SHADES MILL

3.4



3.3.4 Test Species

Juvenile rainbow trout in the parr phase were selected for testing as recommended by ASTM in Standard Practice for Conducting Bioconcentration Tests With Fishes and Saltwater Bivalue Molluscs (125). The species is readily available throughout the year from commercial hatcheries and is easily maintained under laboratory conditions on pelletized food. In this research, the fish were obtained from Gossens Trout Farm, Otterville. The fish were certified free of 13 specific pathogens.

3.3.5 Size of Fish

One to two gram rainbow trout were used in the experiment to minimize the level of organic contaminants in the test fish prior to exposure to the raw drinking water supply. Due to their young age and limited prior exposure they were expected to contain lower levels of organics than larger fish. Since the levels of organic compounds in the water were anticipated to be low, this lower level of initial tissue residue would facilitate analysis of net accumulation of organic compounds beyond the variability of the analytical procedures. Furthermore, small fish have faster uptake rates than large fish and older fish have variable tissue lipid content and possible residue accumulations (60, 70, 109).

3.3.6 Fish Food

Commercial fish food formulations contain measurable concentrations of chlorinated organics, to levels of 400 ug/kg according to Rundel Feed Mills, Palmerston, Ontario (126). These contaminants enter the fish feed through the fish oil (eg. 1400 ug/kg) (6-10%) and fish meal (eg. 300 ug/kg) (20%) fractions of the formulation. In order to eliminate uptake of organics by the fish from the food, Mr. C.Y. Cho, a fish nutritionist was retained to prepare a nutritionally-balanced semi-purified trout food (Table 3.4) similar to those used in fish nutrition research (127, 128, 129, 130). Organic contaminants were reduced to non detectable levels in the semi-purified diet by replacing the fish oil and fish meal constituents with vegetable oils and casein (Tables 3.8, 3.10, 3.11).

TABLE 3.4: FISH FOOD INGREDIENTS[1]

Ingredient	Percent
Casein, vitamin-free	40
Skim milk	11
Soybean protein conc.	10
Dextrin, white	12.8
Wheat middlings	6.0
Vitamin premix)	
Mineral premix)	8.2
DL-methionine)	
Vegetable oil	12

[1]Prepared by C.Y. Cho, 9 March 1980 (Formula C-053)

3.3.7 Procedures

Fish

A six-day period was used at the hatchery to wean the fish from their commercial fish diet to the semi-purified food. The fish were then introduced into the exposure tanks at the two experimental sites on 2 April 1980 when the 12 week exposure commenced. No acclimation period was provided in the experiment as bioconcentration may occur rapidly and steady-state between uptake and clearance can occur within the intial 10 days of exposure (53). The fish were introduced into exposure tanks at the same water temperature as the hatchery. Some appreciation of the practical nature of monitoring was afforded by allowing the water temperature to gradually elevate through the spring and early summer months. However, the temperature was kept within limits of the temperature preferendum for rainbow trout. Temperature control was maintained to ensure that fish at the two sites bioconcentrated the organics at the same rates relative to ambient levels and that bioconcentration occurred at a reasonable rate since the process is closely associated with the metabolic regime of fish.

Five hundred trout fry were held in each of four 100 litre aquaria at each site. The fish were held in replicate aquaria to minimize the possibility of losing the stock from disease or mechanical problems. Each aquarium received a constant flow of water at 1.75 L/min which provided a 95% molecular replacement time of three hours. During the experiment the fish were fed the semi-purified diet twice daily at both sites at a daily rate of 4% of wet body weight. Incandescent lighting simulated a natural 10 h day/14 h night photoperiod. Black shrouds of plastic surrounded the exposure tanks to minimize disturbance.

The fish sampling schedule is shown in Table 3.5. Four fish samples were collected at the hatchery from the stock used for the experiment to determine background levels of organics. Each sample contained 35 randomly selected trout (about 50 g of tissue). One sample was also collected from

TABLE 3.5: FISH SAMPLING SCHEDULE

Sampling Period	Experimental Site Brantford		Reference Site	
	Samples	Fish Weight grams	Samples	Fish Weight grams
0[1]	4 Composites[2]	1.4	4 Composites	1.4
1	" "	1.6	" "	1.6
2	" "	2.0	" "	2.0
4	" "	2.8	" "	2.6
8	" "	4.6	" "	4.8
12	" "	7.3	" "	6.7

[1]initial sampling at hatchery
[2]each composite sample consisted of 35 fish

each of the four exposure aquaria at both laboratory sites after 1, 2, 4, 8 and 12 weeks. Each sample was placed in a glass jar previously cleaned with four glass-distilled solvents and immediately frozen and sent to Ontario Research Foundation (ORF) for organic residue analysis. Table 3.6 presents the analytical scheme as not all samples provided to the ORF were analyzed. Samples of each of the two batches of fish food used during the experiment were also submitted for organic analysis. Fish feeding ceased 24 h prior to each fish sampling period to ensure that the fish food had been either substantially digested or voided.

Resin Cartridges

Cartridges containing adsorbent Amerlite XAD-2 macroreticular resin were used to concentrate suspected organic compounds in the incoming raw water. XAD resin has been successfully employed by other researchers for extraction and recovery of organic pollutants (131, 132, 133, 134). Its large effective surface and wide range of surface polarities, porosities and pore sizes creates high affinity for many organic compounds.

The resin cartridges were installed according to guidelines established by Health and Welfare Canada (135). Prior to use, the resin was cleaned according to procedures outlined by ORF (Table 3.7). After cleaning and packing the resin into a cartridge, ultra pure acetone was passed through the column and analyzed as a blank to assess resin purity. When the resin was shown to be clean it was used in the experiment.

During the experiments, exposure water was passed through each column at a rate of about 15 ml/min. The resin cartridges were renewed at both sites every 1 to 2 weeks. Synchronization of cartridge replacement with fish sampling was maintained. Following removal of a used cartridge, the resin was removed from the column into solvent washed jars with teflon cap liners and then covered with acetone. The glass fiber material used to confine the resin in the cartridges was also stored in jars under acetone. These samples were refrigerated and stored in the dark until analyzed.

TABLE 3.6: BIOACCUMULATION ORGANIC ANALYSES SCHEME

Type of Sample	Brantford	Shades Mill
Fish Tissue	Wk. 0	Wk. 0
	Wk. 2	-
	Wk. 8	-
	Wk. 12	Wk. 12
Resin	Wk. 2	-
	Composite*	Composite*
Fish Food	FF1 + FF2	
	Food common to both sites	

*Sample comprised of all resin samples for that site

TABLE 3.7: ORF PROCEDURE FOR SOLVENT CLEAN-UP of XAD-2 RESIN

SOXHLET EXTRACTION PROCEDURE

1. Acetone : MeOH (80:20) - 12 hours
2. Methylene chloride - 12 hours
3. Hexane - 12 hours
4. Diethyl ether - 4 hours

The resin is removed from the Soxhlet and stored under acetone.

Blanks are determined by either one of the following procedures:

(i) Slurry the resin (known weight) in a beaker with a known volume of solvent. Filter and concentrate the solvent to near dryness, and make up to known volume (1 ml. A suitable aliquot (2 ml) is then taken for GC analysis.

(ii) A small column of the resin is prepared and eluted with a known volume of solvent. The solvent is treated as above prior to GC analysis.

If the blank chromatographic profiles are not satisfactory, additional Soxhlet extraction must be performed.

Water samples were collected throughout the 12 week experiment in 250 ml acid-washed polyethylene bottles for anion and cation analysis. These analyses served to assess the degree of fluctuation of water quality during the research.

Sample Preparation and Analytical Procedures

The analyses performed at ORF involved class selective type analyses for specific target compounds and involved tailored cleanup and analytical determinations for the compounds (Appendix 1) (136).

The specific chemical groupings analyzed in this research were:

1. Polycyclic aromatic hydrocarbons, aromatic hydrocarbons;
2. Organochlorine pesticides, PCB's, chlorinated benzenes;
3. 2, 4-D, 2, 4, 5-T acid herbicides;
4. Chlorinated phenolics (penta-, tetra- and trichlorophenol);
5. Phthalate esters;
6. Organophosphorus pesticides and triazines;
7. Aliphatic and alicyclic organo-halogen compounds eg. chloroethanes, hexachlorobutadiene;
8. Aromatic organo-halogen eg. chlorotoluenes, chlorostyrenes.

3.4 DISCUSSION OF RESULTS

The results of the organic analysis of fish tissue, fish food and resin extractions are summarized in Tables 3.8, 3.9, 3.10 and 3.11 as given by ORF (136.) In all, twenty-two organic compounds were identified in fish or water. These included six OC pesticides, five PAH's, six chlorobenzenes, two chlorophenols, one triazine herbicide, one phthalate ester and PCB. Of these, fifteen were identified in fish and ten in water representing 30% and 20% respectively of the total number of organics analyzed. The concentrations of organics identified in fish or water were at levels near detection limits: ppt in water and ppb in fish tissue. No organo-phosphorus pesticides, phenoxy herbicides and aliphatic, alicyclic or aromatic organohalogen compounds were detected in either water or fish.

At the end of the 12 week fish exposure the organics identified in fish at the downstream experimental site at Brantford and their concentrations in ug/g were: BHC 0.02, B HCH 0.01, dieldrin 0.01, DDE 0.01, hexachlorobenzene <0.01, PCB 0.06, 1,2,4 chlorobenzene 0.03, 1,2,3 chlorobenzene 0.01, 1,2,3,5,)1,2,4,5 chlorobenzene, <0.01, 1,2,3,4 chlorobenzene <0.01, pentachlorobenzene <0.01, 2,3,4,5 chlorophenol 0.04, fluoranthene 0.0045, benz[a]anthracene 0.0016 and DEHP 0.87. Based on the five fold increase in fish weight over the 12 week period (Table 3.5) increased body residue suggested bioaccumulation of all of these organics except possibly DDE, DEHP and hexachlorobenzene (Table 3.12). In this evaluation, minimum detectable amount was used for the week 0 tissue concentrations when levels were below detection. Absence of these organics in fish food and in baseline (week 0) fish tissue further substantiated bioaccumulation. Only the PAH's fluoranthene and benz[a]anthracene were detected in fish food as well as water and thus some of the accumulated residues for these compounds may have originated from food rather than the water. Some DEHP measured in the fish and resin samples is suspected to have arisen from contamination by plastics used during the chemical analysis. For example, the resin blank contained high amounts (24 ug) of this widely-used plasticizer.

TABLE 3.8: RESIDUES IN FISH AND FISH MEAL[a]

Compounds Investigated[b]	Residue Levels in ppm (µg/g wet weight basis)									
	Minimum[c] Detectable Concentration	Fish Meal 2076		Baseline Fish: Time 0		BRANTFORD			12-Week Fish	
		FF1	FF2	1 of 8	8 of 8	2-Week Fish	8-Week Fish		Shadesmill A	Brantford B
Organochlorine Pesticides:										
α-HCH (BHC)	0.002	ND[f]	<0.01	ND	ND	0.02	0.02	0.02	<0.01	0.02
β-HCH	0.002	<0.01	<0.01	ND	ND	<0.01	<0.01	<0.01	<0.01	0.01
γ-HCH (Lindane)	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
p,p'-DDE	0.002	ND	ND	0.02	0.04	0.02	0.01	0.01	0.01	0.01
p,p'-TDE (DDD)	0.004	ND	ND	<0.01	ND	ND	ND	ND	ND	ND
p,p'-DDT	0.004	ND	ND	<0.01	ND	ND	ND	ND	ND	ND
HE	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dieldrin	0.002	ND	ND	ND	ND	ND	0.01	0.01	<0.01	0.01
Endrin	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mirex	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
α-chlordane	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
γ-chlordane	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxy-chlordane	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB:										
(Aroclor 1254)	0.01	0.02	ND	0.04	0.07	0.05	0.05	0.02	0.05	0.06
Chlorobenzenes:										
1,3,5	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4	0.002	ND	ND	ND	<0.01	<0.01	0.01	<0.01	0.01	0.03
1,2,3	0.002	ND	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
1,2,3,5/1,2,4,5	0.001	ND	ND	ND	ND	ND	ND	ND	ND	<0.01
1,2,3,4	0.001	ND	ND	ND	ND	ND	<0.01	<0.01	ND	<0.01
Penta	0.001	ND	ND	ND	ND	ND	<0.01	<0.01	ND	<0.01
Hexa	0.001	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorophenols:										
2,3,4,6/2,3,5,6	0.07	-	-	-	ND	ND	ND	-	ND	ND
2,3,4,5	0.03	-	-	-	ND	ND	ND	-	ND	0.04
Penta	0.03	-	-	-	ND	ND	ND	-	ND	ND
Phenoxy Herbicides:										
2,4-D	0.17	-	-	-	ND	ND	ND	-	ND	ND
2,4,5-T	0.03	-	-	-	ND	ND	ND	-	ND	ND
2,4,5-TP (Silvex)	0.03	-	-	-	ND	ND	ND	-	ND	ND
Organophosphates:										
Diazinon	0.01	-	-	-	-	-	ND	ND	ND	ND
Parathion	0.02	-	-	-	-	-	ND	ND	ND	ND
Malathion	0.06	-	-	-	-	-	ND	ND	ND	ND
Fenitrothion	0.02	-	-	-	-	-	0.05*	0.04*	0.04*	0.04*
Fenitro-oxon	0.04	-	-	-	-	-	ND	ND	ND	ND
Triazines:										
Atrazine	0.10	-	-	-	-	-	ND	ND	ND	ND
Simazine	0.06	-	-	-	-	-	ND	ND	ND	ND
% Moisture	-	10.1	8.2	78.9	79.6	80.3	79.3	-	74.3	74.6
% Fat	-	1.2	1.2	4.7	3.0	3.7	4.7	-	5.9	5.8

(a) Analyses were carried out by capillary GC/EC except for OPs and triazines which were analyzed by packed column GC/N-P. Analysis not carried out is indicated by a dash (-). Values given are the means of duplicate analyses. Values shown with an asterisk (*) indicate that there could be some question about the identity of the residues since the retention times of the GC peaks were slightly off from the standards.

(b) Screening was carried out for other common compounds, e.g. aldrin, photomirex, etc., but except as indicated under the individual samples, none was detected. A number of unidentified peaks (UIPs) were observed and these are referred to by their relative retention times (RRT) to DDE (for OCs) and parathion (for OPs) under the individual samples.

(c) These are based on 2.5 g aliquot fish and recorder peak height response of 1 cm (~ 2 x noise level) when 3 uL injected from a 10 mL volume. Levels below these would not be detected. For practical purposes, values lower than 0.01 ppm are not reported since confirmation is very difficult (if not impossible) at such levels.

(d) Two small UIPs at RRT (relative to parathion) of 0.91, 0.94 observed in sample.

(e) Three small UIPs at RRT (relative to parathion) of 0.41, 0.67, 0.93 observed in sample.

(f) ND = none detected = < the minimum detectable values given in the Table. [See (c)]. Trace values are given as < 0.01 ppm.

TABLE 3.9: RESIDUES IN WATER (RESIN) SAMPLES[a]

Compounds[b] Investigated	Total Residues (ng/100 L BRANTFORD WATER							
	Minimum[c] Detectable Amount	Resin Blank		Week 2			Week 4	
				Glass Wool Extract[d]	Resin Extract		Glass Wool Extract	Resin Extract[e]
		Neutrals	Acidics		Neutrals	Acidics		
OC Pesticides:								
α-HCH (BHC)	20	ND[f]	-	ND	ND	-	-	-
β-HCH	20	ND	-	ND	ND	-	-	-
γ-HCH Lindane	16	18	-	ND	ND	-	-	-
p,p'-DDE	40	304*	-	ND	275*	-	-	-
p,p'-TDE (DDD)	60	70	-	ND	ND	-	-	-
p,p'-DDT	100	248*	-	ND	ND	-	-	-
HE	28	36	-	ND	35*	-	-	-
Dieldrin	40	102*	-	ND	112*	-	-	-
Endrin	100	ND	-	T[g]	ND	-	-	-
Mirex	100	ND	-	ND	ND	-	-	-
α-chlordane	20	ND	-	22	28	-	-	-
γ-chlordane	20	ND	-	34	33	-	-	-
oxy-chlordane	20	ND	-	ND	20*	-	-	-
PCB:								
Aroclor 1254/1260(1:1)	1,000	ND	-	T	ND	-	-	-
Chlorobenzenes:								
1,3,5	25	ND	-	ND	ND	-	-	-
1,2,4	25	ND	-	ND	ND	-	-	-
1,2,3	25	ND	-	ND	ND	-	-	-
1,2,3,5/1,2,4,5	25	ND	-	ND	ND	-	-	-
1,2,3,4	12.5	ND	-	ND	ND	-	-	-
penta	7.5	ND	-	11*	ND	-	-	-
hexa	7.5	ND	-	ND	ND	-	-	-
Chlorophenols:								
2,3,4,6/2,3,5,6	200	-	ND	-	-	ND	-	-
2,3,4,5	100	-	ND	-	-	ND	-	-
penta	100	-	ND	-	-	625	-	-
Phenoxy Acid Herbicides:								
2,4-D	500	-	ND	-	-	ND	-	-
2,4,5-T	100	-	ND	-	-	ND	-	-
2,4,5-TP (Silvex)	100	-	ND	-	-	ND	-	-
Organophosphates:								
Diazinon	80	-	-	-	-	-	ND	ND
Parathion	100	-	-	-	-	-	ND	ND
Malathion	380	-	-	-	-	-	ND	ND
Fenitrothion	120	-	-	-	-	-	ND	ND
Fenitro-oxon	240	-	-	-	-	-	ND	ND
Triazines:								
Atrazine	600	-	-	-	-	-	ND	35,000
Simazine	380	-	-	-	-	-	ND	ND

(a) Analyses were carried out by capillary GC/EC except for OPs and triazines which were analyzed by packed column GC/N-P. Analysis not carried out is indicated by a dash (-). Values shown with an asterisk (*) indicate that there could be some question about the identity of the residues since the retention times of the GC peaks were slightly off from the standards.

(b) Screening was carried out for other common compounds, e.g. aldrin, photomirex, etc., but except as indicated under the individual samples, none was detected. A number of unidentified peaks (UIPs) were observed and these are referred to by their relative retention times (RRT) to DDE (for OCs) and parathion (for OPs) under the individual samples.

(c) These are based on a recorder peak height response of 1 cm (~ 2 x background noise) when 3μL injected from a 10 mL volume. Depending on the background, some lower responses can sometimes be observed and these are indicated by Ts (trace).

(d) The following UIPs with RRT_{DDE} of 0.75, 0.80, 0.82, 0.84, 1.10, 1.28 (_ more significant) were observed. Sample also contained UIPs with retention times close to DDE (67 ng if calculated as DDE), pentachlorobenzene (34 ng if calculated as such), and transnonachlor (24 ng if calculated as such).

Fairly large UIPs at 0.78, 0.85 and 1.28 RRT_{DDE} were also observed in the Florisil blank.

The Florisil blank also contained UIPs with retention times close to DDE (53 ng if calculated as DDE), pentachlorobenzene (18 ng if calculated as such), and transnonachlor (20 ng if calculated as such).

(e) Small UIPs were observed at 0.80, 0.97, 1.18 $RRT_{Parathion}$.

(f) ND = none detected = < the minimum detectable amounts given in the Table [see (c)].

(g) T = trace [see (c) above].

TABLE 3.10: POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN FISH MEAL, FISH, WATER AND PARTICULATE SAMPLES

Compounds Investigated (retention times)	Fish Meal and Fish (µg/g wet weight basis)							*Water Samples (µg/L)			
	Fish Meal		Baseline Fish Brantford	2 week Fish Brantford	8 week Fish Brantford	12 week Fish Brantford	12 week Fish Shades Mill	Resin Blank (Neutrals)	BRANTFORD 2wk Resin (Neutrals)	BRANTFORD 2wk Glass Wool (Brt)	Shades Glass Wool (SM) 2wk.
	FF1	FF2									
Fluoranthene (11.30)	0.0066	0.0091	0.003	0.0015	0.0079	0.0045	0.0014	ND	0.0024	0.0031	0.0062
Benz[a]anthracene (15.18)	0.0386	0.0052	ND	ND	0.0013	0.0016	ND	ND	0.0005	0.0014	0.0013
Benzo[k]fluoranthene(21.13)	0.0073	0.0137	ND	ND	ND	ND	ND	ND	0.0003	0.0012	0.0007
Benzo[a]pyrene (23.51)	0.0015	ND	ND	ND	ND	ND	ND	ND	0.0004	0.0020	ND
Benzo[ghi]perylene(33.07)	0.0074	ND	ND	ND	ND	ND	ND	ND	0.0002	0.0007	ND
	Retention times of other components giving a fluorescence response (not identified)										
	3.41(s)	3.58(s)				3.31(s)	3.30(s)		2.39(vs)	3.89(s)	3.66(s)
			4.78(w)	4.81(s)	4.11(s)	4.79(s)	4.94(s)				
	5.54(s)	5.34(s)	5.28(s)	5.28(s)	5.64(s)		5.28(s)		5.55(vs)		5.39(w)
	6.51(vs)	6.54(vs)							6.41(s)		
					7.34(s)	8.84(s)			7.36(s)	7.80(vs)	7.15(w)
					10.77(s)				7.80(s)		
		12.31(s)							8.54(s)	15.42(w)	
Legend				14.91(w)					11.73(w)	18.62(s)	
w = weak									14.32(s)	19.47(s)	
s = strong	16.07(vs)	16.07(vs)	15.87(w)	15.91(w)	15.84(vs)				19.02(s)	25.05(s)	
vs = very strong			20.85(w)	20.97(w)		20.61(s)	20.81(s)		19.74(s)	34.14(s)	
									21.99(s)	37.16(s)	
										40.46(s)	

* Water (100L) through XAD-2 Resin

TABLE 3.11: ORGANICS IN FISH MEAL, FISH WATER AND PARTICULATE SAMPLES

Compounds Investigated	Fish Meal and Fish (µg/g wet weight basis)							Water Samples (µg/L)		
	Fish Meal		Baseline Fish Brantford	2 week Fish Brantford	8 week Fish Brantford	12 week Fish Brantford	12 week Fish Shades Mill	Resin Blank (Neutrals)	BRANTFORD 2 WEEK Resin (Neutrals)	Glass Wool (Brt)
	FF1	FF2								
Diethylphthalate	ND	ND	Tr	Tr	Tr	Tr	Tr	-	ND	ND
Dibutylphthalate	ND	ND	*Tr	Tr	Tr	Tr	Tr	-	Tr	Tr
Di-2-ethylhexylphthalate	0.27	0.07	0.60	1.40	0.27	0.87	0.33	24 µg	0.05	0.32
Dinonylphthalate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Butylphthalyl butyl glycolate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
†Hexachloroethane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexachlorobutadiene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Octachlorostyrene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexachlorocyclopentadiene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
**Naphthalene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

† Detection limit for this group ranges 2.5 - 3.5 ng/mℓ

* GC-MS using Selected Ion Monitoring indicates range 10-20 ng/g

** GC-MS using Selected Ion Monitoring did not detect these compounds

The number of organics bioaccumulated in the fish increased with length of exposure so that by the twelfth week twelve compounds had bioaccumulated compared with two and six compounds at exposure weeks 2 and 8 respectively. A longer exposure may have resulted in further bioaccumulation of some of the more persistent and lipophilic organics.

Comparison of tissue residue data for week 12, at Brantford and Shades Mill showed higher levels for Brantford than Shades Mill for 13 of the 15 compounds identified (table 3.17). Five of these compounds were not detected in fish at Shades Mill. This probably reflects the municipal and industrial discharges which exist upstream of Brantford which are not present at Shades Mill.

The ten organics identified in water and their concentrations in ng/L were: α chlordane 0.28, γ chlordane 0.33, pentachlorophenol 6.25, atrazine 350.00, fluoranthene 2.4, benz[a]anthracene 0.5, benzo[k]fluoranthene 0.3, benzo[a]pyrene 0.4, benzo[ghi]perylene 0.2 and DEHP 50. These concentrations were derived by taking the total residues measured for each compound and dividing them by the water volume extracted for each resin preparation. Six of these compounds, the two chlordane isomers, atrazine and three PAH's were detected in water but not found in fish. This was not unexpected for atrazine, due to its low residue-forming potential. Chlordane, on the other hand, is highly bioaccumulative but was detected in both the particulate glass wool sample and the resin and may have been adsorbed on particles and unavailable to the fish. The atrazine level of 35,000 ng for the 100 L of water extracted was the highest level detected in the study and similar to values reported by MOE (146). This concentration is typical for water draining a corn producing region (147).

In this study, the Brantford resin sample for week 2 was analyzed to provide an indication of the maximum organic levels in the water since the sample was collected during the period of runoff and snowmelt. It was anticipated that the increased solids loading would carry coincidentally higher organic content (Figure 3.5). This presumption was supported by Olssen who found that increased water flow from feeder streams into a lake in the spring

TABLE 3.12: TOTAL BODY BURDEN IN BRANTFORD FISH

Compound	Total Body Residues (ug) Week 0*	Week 12**	Ratio (Wk.12/Wk.0)	Week Accumulation Detected
α BHC	ND(<.0028)	.104	>37	2
β BHC	ND(<.0028)	.052	>19	2
dieldrin	ND(<.0028)	.052	>19	8
PCB	.07	.312	5	12
1,2,3 chlorobenzene	<.014	.052	>4	12
1,2,4 chlorobenzene	<.014	.156	>11	12
1,2,3,5/ 1,2,4,5 chlorobenzene	ND(<.0014)	<.073	~ 52	12
1,2,3,4 chlorobenzene	ND(<.0014)	<.073	~ 52	8
pentachlorobenzene	ND(<.0014)	<.073	~ 52	8
2,3,4,5 chlorophenol	ND(<.042)	.208	>5	12
benz[a]anthracen	ND(<.0014)	.012	>8	8
fluoranthene	.0042	.0329	8	12

* based on 1.4 g average fish weight

**based on 7.3 g average fish weight
ND = not detected

TABLE 3.13: BCF OF ORGANICS DETECTED IN FISH EXPOSED TO BRANTFORD/SHADES MILL WATER

Chemical	Log BCF	Exposure Concentration	Exposure Period(d)	Half-Life(d)	Log P	Species	Reference
2,3,4,5 Chlorophenol	3.04(est.)	-	-	-	4.30	Orgns c 8% lipids	138
2,3,4,6 Chlorophenol	1.97	0.8 mg/l	1	-	-	Goldfish	91
Fluoranthene	3.49(est.)	-	-	-	4.90	Orgns c 8% lipids	139
1,2,4 Trichlorobenzene	2.26	2.87 ± 1.08 ug/l	3 - 10	1 - 3	-	-	53
1,2,4 Trichlorobenzene	3.32	-	32	-	4.23	Fathead minnow	52
1,2,4 Trichlorbenzene	2.26	2.87 ± 1.08 ug/l	28	1 - 3	-	Bluegill sunfish	53, 65
1,2,4 Trichlorbenzene	2.95	-	32	-	-	Rainbow trout	52
1,2,4 Trichlorbenzene	3.37	-	32	-	-	Green sunfish	52
PCB Aroclor 1254	4.57	1.0 ug/l	28	42	-	-	53
PCB Aroclor 1254	5.00	-	32	-	6.47	Fathead minnow	52
DDE	4.71	-	32	-	5.69	Fathead minnow	52
DEHP	2.76	1.9 ug/l	56	14.2	-	Fathead minnow .	140
	1.96	62 ug/l	56	18.3	-	Fathead minnow	
1,2,3,5 tetrachlorbenzene	3.26	7.72 ± 0.59 ug/l	28	2 - 4	-	Bluegill sunfish	53
Atrazine	<0.90	-	276	-	2.63	Fathead minnow	52
Chlordane	4.58	-	32	-	6.00	Fathead minnow	52
Pentachlorophenol	1 (muscle)	0.1 mg/l	8	-	-	Bluegill sunfish	141
	2.54 (liver)	0.1 mg/l	8	-	-	Bluegill sunfish	141
Pentachlorophenol	2.89	-	32	-	5.01	Fathead minnow	52
Pentachlorobenzene	3.53	5.15 ± 2.20 ug/l	28	>7	-	Bluegill sunfish	53
Hexachlorobenzene	4.30	5.0 ug/l	14	>60	-	-	53

Turbidity Measurements during Bioaccumulation Exposure

3.5

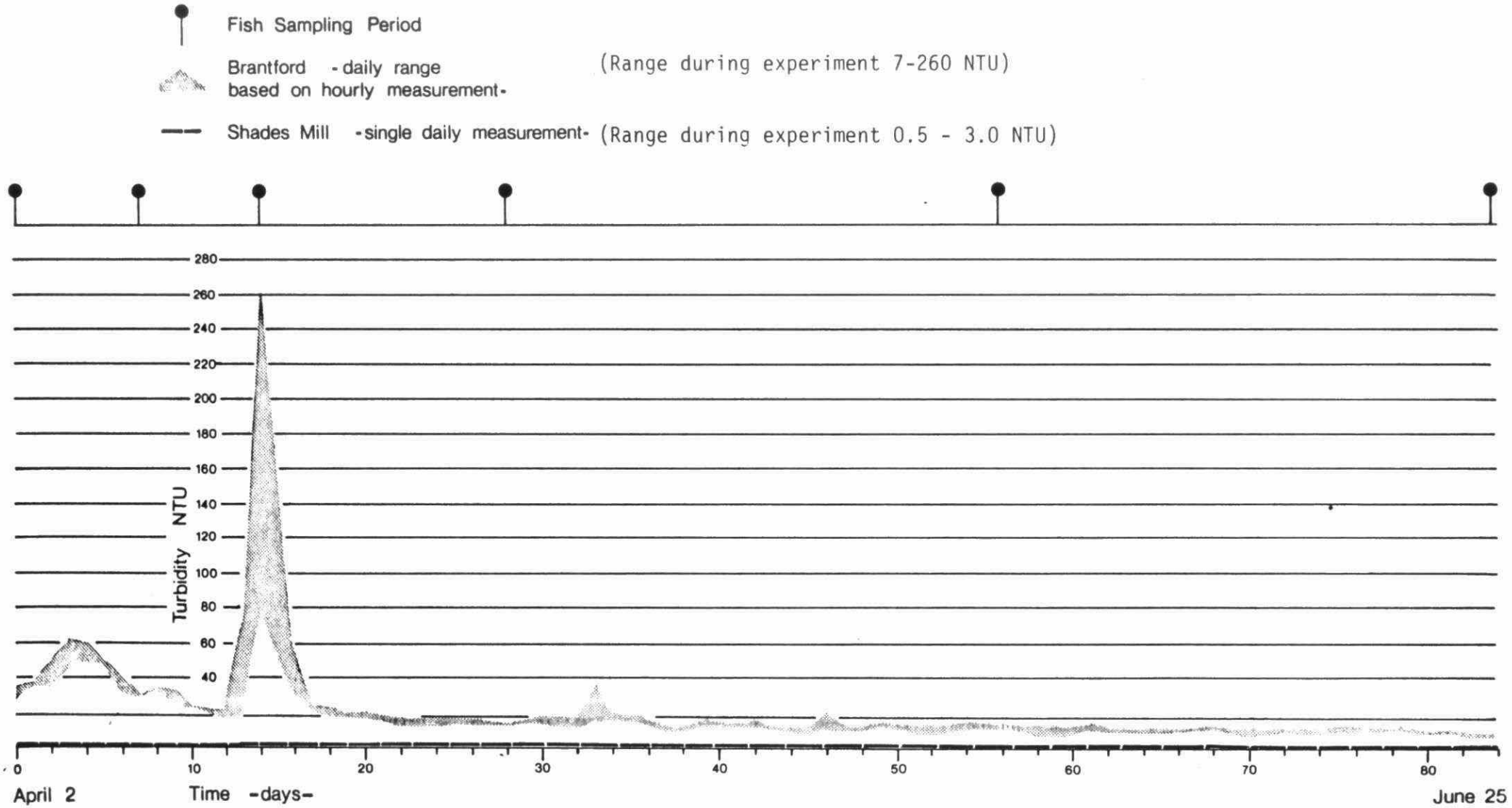


TABLE 3.14: ORGANIC CONCENTRATIONS IN FISH (ug/g, wet wt.)

Compound	IEC[1] Brantford	Poels et al 1978 (138)[2] Groundwater	Poels et al 1978 (138)[2] Rhine River	MOE[3]	Guideline[4]
DDT & Metabolities	<0.02	0.16	0.44	-	5.
PCB's	0.05	1.1	2.0	0.16	2.
Mirex	ND	-	-	ND	0.1
HCB	<0.01	0.46	6.3	-	-
Dieldrin	0.01	<0.05	0.1	-	-
Pentachlorobenzene	<0.01	0.19	2.5	-	-
HCH isomers	≤0.02	ND	ND	-	-

[1] 12 week exposed rainbow trout to Grand River water

[2] 6 month exposed rainbow trout to groundwater and Rhine River water

[3] a 1 kg carp collected 1977 in Grand River near Galt (143)

[4] guideline below which consumption of fish is acceptable (144)

TABLE 3.15: INORGANIC ANALYSIS OF BIOACCUMULATION WATER (mg/l)

Date	Exposure Day	Site	Fe	K	Mg	Mn	Na	Ni	P	Pb	Al	Ba	Ca	Cd	Cr	Cu	Zn
8/4/80	6	Brantford	.81	1.9	9.39	.026	9.0	<.006	0.2	<.01	.76	.016	43.7	<.002	.012	.005	.012
8/4/80	6	Shades Mill	.14	1.2	19.7	.009	8.7	.012	0.2	<.01	.12	.032	56.1	<.002	.022	.006	.020
16/4/80	14	Brantford	2.05	2.1	10.7	.042	11.4	<.006	0.2	<.01	1.84	.023	49.9	<.002	.019	.010	.021
16/4/80	14	Shades Mill	.18	1.6	17.7	.013	7.4	<.006	0.2	<.01	.1	.029	54.1	<.002	.025	.004	.030
4/5/80	32	Brantford	.31	2.1	16.7	.028	19.6	<.006	0.2	<.01	.23	.019	59.1	<.002	.020	.006	.017
4/5/80	32	Shades Mill	.14	1.3	20.4	.016	7.9	<.006	0.1	<.01	.04	.032	60.3	<.002	.027	.006	.025
23/5/80	51	Brantford	.48	2.5	19.1	.048	18.5	<.006	0.2	<.01	.34	.022	67.6	<.002	.024	.012	.025
25/6/80	84	Brantford	.46	2.9	20.0	.058	31.2	<.007	0.2	<.01	.37	.022	60.9	<.002	.023	.014	.037
25/6/80	84	Shades Mill	.14	1.2	23.2	.015	8.6	<.006	0.2	<.01	.07	.038	60.2	<.002	.026	.010	.025

TABLE 3.16: BIOACCUMUALTION STUDY WATER QUALITY CHARACTERISTICS

Parameter	Experimental Site Brantford		Reference Site Shades Mill	
	Mean	Range	Mean	Range
Dissolved Oxygen (mg/L)	8.1	6.2 - 10.00	8.6	6.2 - 10.2
Temperature (°C)	13.3	10.0 - 19.5	12.6	8.7 - 17.5
pH	7.9	7.1 - 8.6	8.0	7.3 - 8.4
Conductivity (umhos)	614	431 - 770	495	442 - 547
Turbidity (NTU)	33	7 - 260	1.8	<1 - 3.4

TABLE 3.17 COMPARISON OF TISSUE CONCENTRATIONS AT EXPOSURE WEEK 12

Compound	Tissue Concentration (ug/g)	
	Shades Mill	Brantford
α BHC	<0.01	0.02
β BHC	<0.01	0.01
dieldrin	<0.01	0.01
PCB	0.05	0.06
1,2,4 chlorobenzene	0.01	0.03
1,2,3 chlorobenzene	<0.01	0.01
1,2,3,5/ 1,2,4,5 chlorobenzene	<0.001*	<0.01
1,2,3,4 chlorobenzene	<0.001*	<0.01
pentachlorobenzene	<0.001*	<0.01
2,3,4,5 chlorophenol	<0.03*	0.04
Benz[a]anthracene	<0.001*	0.0016
fluoranthene	0.0014	0.0045
DDE	0.01	0.01
hexachlorobenzene	<0.01	<0.01
DEHP	0.33	0.87

* detection limit

coincided with the maximum levels of PCB's observed in roach tissue for the entire year (74). However, in the present study, organics levels did not increase markedly during the runoff period in the fish or water.

As nine of the twelve organics accumulated in fish tissue were not detected in water it was not possible to calculate bioconcentration factors for the study. However, using BCF's taken from other studies (Tables 3.2, 3.13) it is clear that using fish as accumulators enabled the detection of organic compounds present in water at levels several orders of magnitude below the detection limits of chemical methods for water analysis. A recent MOE study discussed the accumulation of organics occurring below measurable levels in water (137). Similarly, MOE found that hatchery-reared rainbow trout exposed to diluted industrial effluents for 48 hours accumulated ppb levels of various organics (eg. styrene, naphthalene, benzene, dichlorobenzene, toluene) undetected in the raw effluent.

The levels measured in fish in the IEC study were lower than those found in a recent study of a Rhine River drinking water supply (142). In fact, the levels observed by IEC for fish tissue were markedly below those found in the fish exposed to a groundwater source in the European study (Table 3.14). The levels in fish did not approach guidelines restricting fish consumption and the levels in water were within drinking water guidelines.

Tables 3.15 and 3.16 summarize other water quality characteristics. The level of organics in the Brantford water supply was low and not environmentally significant or of public health risk and were concentrations expected in a watercourse receiving dilute treated municipal and industrial discharges.

3.5 CONCLUSIONS AND RECOMMENDATIONS

This experiment demonstrated the feasibility of using bioaccumulation in fish to monitor organics in drinking water supplies at concentrations below detection limits of conventional water analysis. Fish tissue residues can thus provide a sensitive measure of public exposure to organics and may be included in monitoring programs established to protect the public. Except for the addition of some clean-up steps, fish tissue analysis was carried out with the same ease as water analysis and at a low per capita cost based on the populous protected.

Twenty-two organic compounds were identified in the study, all at levels close to the chemical analysis detection limits. The detection limits were in the parts per trillion range in water and parts per billion range in fish tissue samples. In spite of the greater sensitivity of water analysis, one third more compounds were measured in fish tissue than identified in water samples which were extracted with a polymer resin. Nine of the twelve compounds bioaccumulated in fish were not detected in water. Over the twelve week exposure period, fish accumulated residues of OC pesticides, chlorobenzenes, tetra chlorophenol, PCB and several PAH's. A few compounds detected only in water samples were either non-bioaccumulative (eg. atrazine) or probably adsorbed to organic particles and unavailable for accumulation (eg. chlordane). Therefore, bioaccumulation not only enabled the detection of many trace organics but also established their environmental significance based on resin forming potential, reactivity and stability.

In spite of the low organic concentrations in water, the levels and compounds identified in fish tissue at the developed and undeveloped sites clearly demonstrated differences in organic inputs at the two locations on the river basin. By exposure week 12, fish at the site located downstream of treated industrial and municipal discharge areas showed higher levels for 13 of the 15 compounds identified, five of which were not detected in fish at the upstream reference site. A number of factors may influence bio-

accumulation in fish. For example, the holding of hatching fish of uniform size, age and condition in aquaria receiving aerated, temperature-controlled water at a constant flow rate was essential to demonstrate differences in organic content of the water at the two sites on the river. Moreover, the use of a semi-purified fish food formulation was necessary to reduce the possibility of trace organic uptake from the food.

As the fish exposure progressed, the number of compounds demonstrated to bioaccumulate increased and the tissue concentrations increased. A longer fish exposure may have increased bioaccumulation of the more lipophilic and stable organics. However, this research was not designed to monitor for an extended period.

Three approaches are available to investigate the potential organic contamination of drinking water.

1. Indigenous fish sampling which would have prolonged exposure to organics in water and to food. The collection and analysis of resident fish would provide further information on microorganic concentrations in the river system. The contribution to organic accumulation through the food chain increases with lower levels of organics in water, and with higher trophic status and with more lipophilic and persistent compounds. Interfering factors such as fish health, size, migration and season of the year must be understood and considered in the design of such a monitoring program. Indigenous fish should be used as an overall indicator, not specific for water alone.

2. In situ biomonitoring (caged fish) could be used in areas that are suspected to be contaminated to confirm source and identity of organics.

3. On site biomonitoring under controlled experimental conditions to permit increased sensitivity and to enable comparison of different locations. This research successfully used this approach which enabled demonstration of differences in organic inputs at the two experimental sites.

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4.0 FISH PHYSIOGRAPH

4.1 LITERATURE REVIEW OF FISH PHYSIOGRAPH SYSTEMS

Fish have been used for continuous toxicity monitoring for two decades. The earliest monitoring systems used caged fish exposed to effluents and relied on mortality and signs of stress as toxicity indicators (1, 2). These methods identified sources of acute toxicity but did not allow time for remedial action. Furthermore, these monitoring techniques required the constant presence of an observer. Such systems are still used and one is presently being installed at the Guelph Sewage Treatment Plant (3). However, most systems presently in use monitor pre-lethal toxic symptoms. This approach takes advantage of the long delay which may exist between the onset of toxicity and death and thus enables the detection of lower concentrations of toxics and provides more time for corrective action.

A number of parameters related to fish swimming activity have been used to monitor toxic situations such as: total activity, loss of rheotaxis, avoidance-preference selection and fish movement patterns. For example, total activity has been monitored by recording impulses from a relay connected to paddles deflected by currents produced by the fish (4). Several researchers recorded activity as the frequency of breaks of light beams from photocells aligned across fish tanks (5, 6, 7). Infrared light emitters were recommended instead of visible light beams to avoid disturbance of the fish during the dark cycle (8, 9). However, turbid water may block light beam passage in this form of system.

A number of toxicants have been demonstrated to alter total activity of fish. Changes in fish activity were recorded at zinc levels between 2.94 and 3.64 mg/L in 96 hour exposures by making hourly comparison of changes in the variance of automatically compiled photocell interruptions (7). Similarly, locomotor activity of bluegill has been used to monitor sublethal concentrations of DDT, chromium, cadmium and zinc (10, 11).

In another study, alterations in fish activity were shown to occur at sub-lethal concentrations of a munitions plant effluent (12, 13, 14). The system used was capable of automatically transferring data on light beam interruptions to a minicomputer to be later compared to pre-determined upper and lower 95% confidence limits to delineate abnormal activity.

An electronic system has been developed by Morgan which uses a maximum 99% confidence limit to define normal activity for each fish and it uses integrated alarms that activate if these pre-set values are exceeded (9). The responsiveness of this system to seven laboratory-tested toxicants was similar to that found using a system based on respiratory activity (9, 15).

Fish movement patterns have also been monitored using photocells. The photocells when continuously interrupted designated the position of the fish in the test chamber. A minicomputer coupled to photocells has been designed to provide detailed data evaluation and to demonstrate a response to gradients of copper (16, 17). Along a similar line, an automated technique has been developed using infrared emitters to demonstrate avoidance reactions of pinfish (Lagoden rhomboides) to 0.02-0.04 mg/L of chlorine produced oxidants (8). These systems had the problem of dealing with large amounts of data generated in a short period.

Fish have also been shown to avoid such contaminants as zinc and various pesticides (18, 19). However, preference-avoidance responses have been shown to vary widely for different compounds. In one study, green sunfish avoided only 8 of 40 compounds tested (20). Changes in movement pattern were complex, not easily analyzed and therefore not amenable for rapid monitoring (9, 21).

Loss of rheotaxis or the ability of a fish to maintain position in a current has been used to monitor a number of toxicants, such as, fenitrothion, pulp fiber, pulp mill effluent and detergents (22, 23, 24, 25). Photocells and an electric shock mechanism has been used to monitor loss of rheotaxis (26). If 2 of 3 fish spent more than 5 of 15 minutes in the downstream end of fish

channels, an alarm was activated. Used for 18 months to monitor Rhine River water, no alarm responses occurred although there were a few operational difficulties. A similar system was used to detect sublethal levels of phenol and DDT at acutely toxic levels (27). This system used alternating periods of quiescency and swift currents. A kinetic screen provided a measure of time spent in the downstream section of the fish channels by producing electrical impulses when contacted.

Another means of testing the ability of a fish to maintain position is to place the fish in a confining tube through which flow rate is maintained and the tube is rotated. At some speed of rotation, the fish no longer compensate and begin to rotate. This technique has been used to demonstrate differences between fish exposed or not exposed to zinc or methylmercury (28, 29). This method has only limited application as a continuous monitoring system due to the frequent recovery periods required and because of automation difficulties.

Heart rate has also been suggested as a parameter to monitor toxic conditions. Although early methods used cannulae and had to deal with problems of stress due to the surgical procedure and restricted movement, methods of remote sensing are now available (30, 31, 32). In one study, enhanced heart rate was observed in rainbow trout exposed to sublethal levels of DDT although dieldrin induced changes only at lethal levels (33).

Changes in blood composition such as elevated glucose, cortisol and blood pH and O_2 have been suggested as measures of toxic stress (34, 35, 36, 37). Technical difficulties from automatic sampling and analysis have restricted their use in continuous toxicity monitoring systems.

Changes in oxygen consumption rates also indicate toxic effects. Toxicants shown to affect oxygen consumption include benzene, methoxychlor, copper and BKME (38, 39, 40, 41). Two techniques suitable for automation have been used: oxygen elctrodes and electrolysis cells. One drawback of the oxygen monitoring technique is the necessity of stopping water flow when assessing

oxygen consumption. In addition, compensation for variables which affect oxygen consumption such as temperature must be taken into account during monitoring.

Ventilatory parameters such as ventilatory rate, gill purge rate and depth of ventilation have been used extensively to monitor toxicity. Rapid opercular movement from cough or gill purges result in reversal of water flow over the gill surface (42,43). Techniques to monitor fish ventilatory movements have been reviewed by Heath (44).

Research suggests that monitoring using fish respiratory parameters is among the most sensitive (45, 46, 47). Signs of respiratory impairment appear during exposure to sublethal concentrations of a number of contaminants and in sufficiently short time to allow remedial action.

Tables 4.1, 4.2 and 4.3 present a summary of reported responses of fish to various organics, heavy metals and miscellaneous compounds. Toxicant levels of 5 to 30% of the 48 h LC50 or 20% to 50% of the 96 hr LC50 evoked significant changes in respiratory pattern within 24 hours. Lethal levels were detected within several hours. For some compounds such as lindane, malathion, lead and copper, short-term monitoring of respiratory activities provided as sensitive an indicator as long-term studies using growth or reproductive impairment criteria (51). Unlike various behavioural or locomotory responses, changes in respiratory rate, increased gill purge response and changes in depth of respiration monitor the strength of the stimulus. These responses can be measured easily and quantified electronically.

The development of systems to monitor fish respiratory responses commenced more than a decade ago. The first studies to demonstrate correlation between respiratory impairment and toxic exposure used cannulated fish (30, 41, 63, 64). Strong millivolt signals associated with ventilatory movements and gill purge response were recorded on strip charts and evaluated manually. Although effective in delivering clear signals, the technique

TABLE 4.1: REVIEW OF DETECTION LIMITS OF BIOMONITORING SYSTEMS USING FISH RESPIRATORY RESPONSES: ORGANICS

Class of Organics	Effective (mg/L)	Concentration (LC50 or MATC)	Time to Response (hr)	Response	Test Species and Size	Method	Reference
Aromatics							
Naphthalene		30% 96 h	3	>ventilatory & gill purge rate	pink salmon fry 5 cm	non invasive electrodes	(48)
Toluene	2.5	10% 48 h	<24	>ventilatory rate	rainbow trout 56 g.	non invasive electrodes	(49)
Xylene	2	10% 48 h	<24	"	"	"	"
OC Pesticides							
Chlordane	0.004	1% 48 h	840	>ventilatory rate	largemouth bass 103 g.	"	(50)
"	0.03	10% 48 h	33	"	"	"	"
"	0.05	20% 48 h	<24	"	"	"	"
"	0.27	100% 48 h	10	"	"	"	"
pp' DDT	0.002		<72	>gill purge rate	brook trout	non invasive electrodes	(51)
"	0.005	10% 48 h	>96, <168	"	coho salmon	cannulae	(52)
"	0.020	25% 48 h	<120	"	"	"	"
"	0.050	100% 48 h	3	"	"	"	"
"	0.053-0.140	200% 48 h	<5	>ventilatory & gill purge rate	rainbow trout 120 g.	"	(33) "
Dieldrin	0.125-0.250	10% 96 h	0.25	>gill purge rate	"	"	"
Endrin	0.0004	100% 96 h	240	"	bluegill	non invasive electrodes	(51) "
"	0.006	10% 96 h	<24	"	"	"	"
Lindane	0.006	MATC 0.009-0.017	<72	"	brook trout	"	"
OP PESTICIDES							
Diazinon	0.025	MATC 0.008	<72	"	"	"	"
Fenitrothion	0.23	18% 96 h	<2	"	coho salmon 0.4-5.5 g.	observed visually	(53) "
"	0.48	37% 96 h	<2	"	"	"	"
Malathion	0.007	MATC <0.016	<72	"	brook trout	non invasive electrodes	(51)
MethylParathion	0.007	1% 48 h	840	15% >ventilatory rate	largemouth bass 103 g.	"	(50)
"	0.05	10% 48 h	41	>ventilatory rate	"	"	"
"	0.1	15% 48 h	<24	"	"	"	"
"	0.56	100% 48 h	<24	"	"	"	"
Halogenated Alkanes and Cycloalkanes							
Carbon Tetrachloride	2.6		3-6	>ventilatory amplitude	bluegill 8 g.	"	(55)
"	3.7		"	>amplitude & ventilatory rate	"	"	"
Chloroform	20.8		"	2 X <amplitude	"	"	"
"	37.9		"	<amplitude & ventilatory rate >gill purge rate	"	"	"
-Hexachlorocyclohexane	0.04	33% 48 h	<24	>ventilatory rate	rainbow trout 56 g.	"	(49)

TABLE 4.1: REVIEW OF DETECTION LIMITS OF BIOMONITORING SYSTEMS USING FISH RESPIRATORY RESPONSES: ORGANICS

Class of Organics	Effective (mg/L)	Concentration (LC50 or MATC)	Time to Response (hr)	Response	Test Species and Size	Method	Reference
Halogenated Alkenes							
Hexachlorobutadiene	0.05	5% 48 h	<24	>ventilatory rate	rainbow trout 56 56 g.	non invasive electrodes	(4
Tetrachloroethylene	2.1		3-6	>ventilatory & gill purge rate 2 X 6 amplitude	bluegill 8 g.	"	(55)
"	4.0		3-6	"	"	"	"
Trichloroethylene	5	8% 48 h	<24	>ventilatory rate	rainbow trout 56 g.	"	(49)
Halogenated Benzenes							
O/M Dichlorobenzene	0.5	5% 48 h	"	"	"	"	"
Phenolics							
Phenol	4	7% 48 h	"	"	"	"	"
"	1		11	"	rainbow trout	"	(55)
"	5		1	"	"	"	"
"	0.5	2.5% 48 h	<48	"	largemouth bass	"	(1
"	1.0	10% 48 h	<24	"	yellow perch	"	"
"	1.0	5% 48 h	<24	"	largemouth bass	"	"
"	1.5	10% 48 h	<24	"	Mossambique bream	"	"
Pentachlorophenol	0.005	1% 48 h	840	"	largemouth bass	"	"
"	0.04	10% 48 h	74	"	"	"	"
"	0.1	15% 48 h	<24	"	"	"	"
"	0.38	100% 48 h	12	"	"	"	"
"	0.07	17% 48 h	<24	"	rainbow trout 56 g.	"	(49)
Surfactants							
C LAS	<2.19	0.70<MATC<1.1	<120	mean ventilatory rate for 24 h periods over 5 days	bluegill 8 cm	"	(5
C LAS	<0.39	0.10<MATC<0.24	"	"	"	"	"
Alkyl Ethoxylate Sulfate	<0.39	0.13<MATC<0.20	"	"	"	"	"
C Alkyl Ethoxylate	>1.56	MATC>1.56	"	"	"	"	"
C Alkyl Ethoxylate	<0.54	0.32<MATC<1.0	"	"	"	"	"
Amine Oxide	<2.99	0.50<MATC<1.0	"	"	"	"	"
NTA	>181	MATC>54	"	"	"	"	"
Organo Nitrile							
Acrylonitrile	5	33% 48 h	<24	>ventilatory rate	rainbow trout 56 g	"	(49
Carbamate Pesticide							
Sevin	0.09	1% 48	840	15%>ventilatory rate	largemouth bass	"	(15
"	0.81	10% 48	26	>ventilatory rate	"	"	"
"	1.0	15% 48	<24	"	"	"	"
"	2.00	30% 48	0.5	>gill purge rate	rainbow trout 120 g.	cannulae	(33
"	9.84	100% 48	8	>ventilatory rate	largemouth bass	non invasive electrodes	(15
"							
Herbicide							
Diquat	1	10% 96	24	>gill purge rate	yellow perch 2 year old	buccal catheter pressure transducer	(57)
"	5	50% 96	10	"	"	"	"

TABLE 4.2: REVIEW OF DETECTION LIMITS OF BIOMONITORING SYSTEMS USING FISH RESPIRATORY RESPONSE: HEAVY METALS

Compound	Effective (mg/L)	Concentration (LC50 or MATC)	Time to Response (hr)	Response	Test Species	Method	Reference
Cadmium	0.005	MATC	<72	>gill purge rate	brook trout	non invasive electrodes	(51)
"	0.010-0.030		<14	>ventilatory rate	rainbow trout	"	(58)
"	0.025		<24	"	"	"	(49)
"	0.15	10% 48 h	<24	"	largemouth bass	"	(46)
Copper	0.09-0.015	MATC	5	>gill purge rate	brook trout	"	(51)
"	0.048	5% 48 h	<24	>ventilatory rate	largemouth bass	"	(46)
"	0.06		"	"	rainbow trout	"	(49)
Lead	0.080	MATC	<72	>gill purge rate	brook trout	"	(51)
"	1.05	10% 48 h	<24	>ventilatory rate	largemouth bass	"	(46)
Methylmercury	0.003	MATC	<72	>gill purge rate	brook trout	"	(51)
Mercury	0.01	5% 48 h	<24	>ventilatory rate	largemouth bass	"	(46)
Zinc	0.53		<24	>ventilatory rate <signal amplitude	bluegill sunfish	"	(59)
"	1.39	MATC 0.53-1.37	<72	>gill purge rate	brook trout	"	(51)
"	2.55		52	>ventilatory rate	bluegill sunfish	invasive & non invasive electrodes	(30)
"	4.16		8	"	"	"	
"	3.64		96	fish movement	"	light beam interruption	(7)
"	3		2	>gill purge rate	"	cannulae	(30)
"	6		2	"	"	"	"
Dichromate	0.860	MATC 0.20-0.35	<72	>gill purge rate	brook trout	"	(51)

TABLE 4.3: REVIEW OF DETECTION LIMITS OF BIOMONITORING SYSTEMS USING RESPIRATORY RESPONSES: MISCELLANEOUS COMPOUNDS

Compound	Effective (mg/L)	Concentration (LC50 or MATC)	Time to Response (hr)	Response	Test Species	Method	Reference
Ammonia	0.11 (free)		2	>ventilatory rate	rainbow trout	non invasive electrodes	(55)
"	5.01 (as N)	30% 48 h	<24	"	largemouth bass	"	(46)
BKME		20% 96 h	3	>gill purge rate	sockeye salmon	cannulae	(41)
Chlorine	0.06 (TRC)		<1	>ventilatory rate	bluegill sunfish	non invasive electrodes	(60)
Cyanide	0.01	10% 48 h	<24	"	largemouth bass	"	(46)
Pulp Mill Effluent (3)	5.6% effluent concentration (secondary or equivalent treated)		<24	>gill purge rate	bluegill sunfish	non invasive electrodes	(51)
Carpet Mill Effluent	5.6% effluent concentration		<24	"	bluegill sunfish	"	"
Chlorinated Municipal Effluent	5.6% effluent concentration		<24	"	bluegill sunfish	"	"
Chemical Plant Effluent (3)	5.6% effluent concentration		<24	"	bluegill sunfish	"	"
Coal Mine Effluent	5.6% effluent concentration		<24	"	bluegill sunfish	"	"
Uranium Mine Effluent	5.6% effluent concentration		<24	"	bluegill sunfish	"	"
Ammunition Plant Wastewater	0.1% effluent concentration		<24	"	bluegill sunfish	"	"
Hydrogen Sulfide	0.3-1.1	-		"	sockeye salmon smelts	cannulae	(61)
Petroleum Refinery Efluent	between 50 and 100% effluent concentration		<3	>ventilatory rate	rainbow trout	non invasive electrodes	(62)
	between 25 and 50% effluent		<3	>gill purge rate	rainbow trout	non invasive electrodes	(62)

had limitations as the surgical procedure stressed the fish and long-term monitoring was not possible due to damage to the fish caused by the implanted electrodes.

Roberts was the first to show that stainless steel mesh electrodes placed at either end of a chamber could detect changes in ventilatory electric potential of unrestrained fish (65). However, the chamber was too restrictive. Other investigators later developed an electrode chamber suitable for monitoring the respiratory responses of unstressed free-swimming fish (66, 67). Conductive electrodes at either end of a holding chamber enabled the electronic sensing of the μ.V. electrical potential associated with muscle movements in the bronchial region of the fish. Continued refinements in the amplifying and filtering capabilities of the signal processing units resulted in reliable, noise-immune amplifiers (68.)

Several continuous monitoring systems have been developed utilizing fish ventilatory parameters. The simplest system used timing devices to control activation of a multi-channel physiograph on which the ventilatory waves were recorded (30, 35, 54, 56, 64, 69, 70). This technique had the disadvantage of requiring tedious manual interpretation of the recordings.

Real-time on-line evaluation of ventilatory rate data was first achieved in 1977 with the development of a hardware based electronic system (15, 46, 71). The module automatically compiled data on rate of respiration for each of twelve fish using electronic peak detection counters, and continuously compared rates for each minute to pre-determined maximum limits. Alarm circuitry was activated whenever a pre-set limit was exceeded. However, this system had problems including an inherent insensitivity in the alarm setting due to their failure to account for diurnal variation, an inability to monitor conditions that depressed respiratory rate, a lack of on-going comparison with control fish and frequent false detection during fish feeding. Basically, the system lacked flexibility.

The recent incorporation of computer logic to biological monitoring systems offers considerable promise in providing the necessary flexibility. Field testing of these systems has begun, however, more research is required (60, 72). Problems of false detections due to environmental influences and short-comings of the system are unresolved. However, major advances have been made and seven early warning systems based on ventilatory responses are being developed (Table 4.4). Each system is a research tool and most are being refined in the laboratory. With the number of systems designs being tested and the proven responses of fish to various toxicants, automated continuous toxicity monitoring, which is sensitive and reliable, should be possible in the near future.

Further detailed discussion of physiograph systems development based on the literature review is contained in Section 4.3.

TABLE 4.4: EARLY WARNING SYSTEMS USING FISH

RESEARCHERS	APPLICATION	KEY REFERENCE
Cairns, Gruber and others VPI & SU	research and effluent monitoring in the field	Cairns and Gruber 1980 (73) Gruber et al 1980 (60) Gruber et al 1978 (74) van der Schalie et al 1979 (72)
Carlson and Drummond US EPA, Duluth	research and effluent evaluation in the laboratory	Carlson 1980 (75) Carlson and Drummond 1978 (70) Drummond and Carlson 1977 (51)
Maki Procter and Gamble Cincinnati	research and dose-response evaluation in the laboratory	Maki 1979 (56)
Miller WRC Stevenage, U.K.	research and effluent monitoring in the field	Miller et al 1979 (55) Miller 1977 (58)
Morgan NIWR Pretoria, South Africa	research and dose-response evaluations in the laboratory	Morgan 1977 (46) Morgan 1975 (50) Morgan and Kuhn 1974 (71)
Neiheisel US EPA, Cincinnati	research on drinking water monitoring using dose-response in laboratory	Capute 1980 (54)
Sloof NIWS Leidschendam, Netherlands	research and dose-response evaluations in the laboratory	Sloof 1979 (49)

4.2 RATIONALE FOR EXPERIMENTAL APPROACH

An automatic technique is needed to rapidly and reliably warn of developing toxic situations in water to prevent ecological damage and protect human health. This is particularly true for waters suspected of containing elevated concentrations of organic compounds since the analytical techniques, concentrations, and biological significance of these chemicals have been only partly established. Water management practices such as multiple use and re-use of water within a watershed coupled with the possibility of accidental contamination have intensified this demand. Automated monitoring systems could be located on drinking water treatment facility inlets and treated effluent outfalls.

Automated physical-chemical monitors can continuously measure a number of parameters. However, they are unable to monitor the quality of complex waters and effluents. Chemical analysis of many key environmental contaminants is costly and time consuming and therefore not routinely undertaken. Although chemical analyses can be compared to regulatory standards, the comparison does not directly establish the toxic potency of water nor does it account for possible interactions of components or effects which may alter the toxicity.

Biological monitoring, used in conjunction with physical-chemical determinations, has potential as an early warning tool. Subtle changes in water quality may be detected by monitoring the response of test organisms which continuously integrate the effects of all parameters. However, not all biological monitoring methods are suitable for such a program. Traditional means of monitoring such as biotic index assessment and acute and chronic toxicity testing are inappropriate primarily due to test time.

Research has shown that monitoring methods using fish respiratory parameters are among the most sensitive (15, 45). Signs of respiratory impairment appear during exposure to sublethal contaminant concentrations and within sufficiently short periods to enable remedial action. Changes in respiratory rate, increased gill purge response and changes in depth of respiration; unlike various behavioural or locomotory responses, monitor the strength of the stimulus, and can be easily measured and quantified electronically.

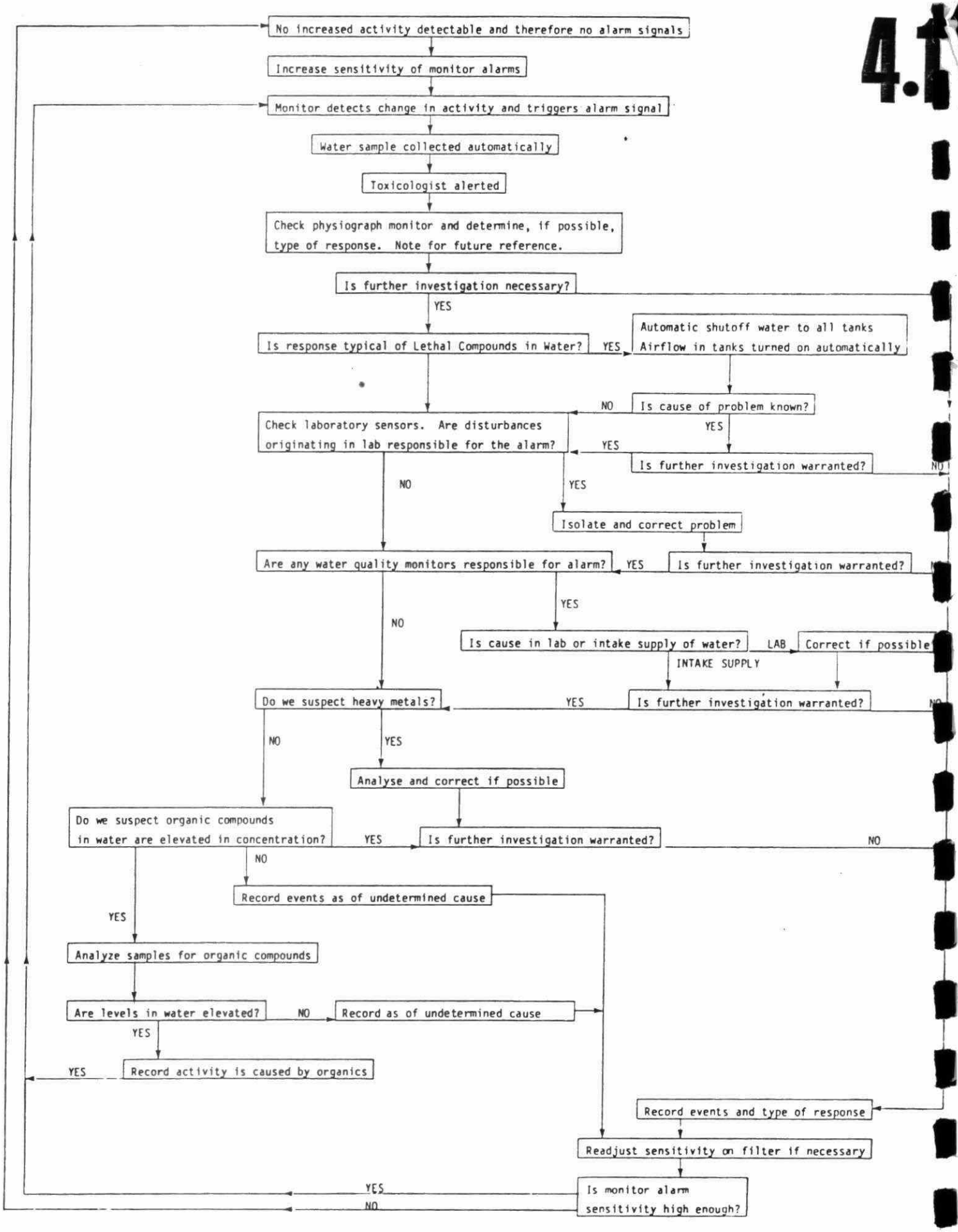
IEC's research presents the preliminary development of such an early warning system to detect elevated levels of organics in water. The research included a review and evaluation of previous fish physiograph systems, the set-up and testing of a hard copy system in a practical field situation and the results of the first dose-response applications in Canada. The installed system was the first in the world to monitor a public water supply.

The acute physiograph study had two phases. The initial phase was a feasibility study to determine if fish respiratory activity could be used to monitor organic compounds in a drinking water source. This represented a new field application as the fish physiograph approach had never been applied to monitoring the variations in water quality of raw drinking water. This research was also state-of-the-art in its attempt to discriminate responses due to one class of compound.

The first phase of the study included a detailed review of previous physiograph systems as background for design of the experimental unit followed by its set-up, calibration and tuning. Experiments were then undertaken to determine if changes in fish activity occurred during exposure to the untreated drinking water.

A hard copy recording system was used to enable full inspection of the respiratory waves so that several respiratory parameters could be assessed and a stable, predictable output could be established. This was a necessary step if the system was to be computer automated for practical monitoring. Electrodes, electrode chambers and amplifier/filters compatible with an automated approach were used.

The procedure involved a step-wise approach to continuously improve the sensitivity of the physiograph to detect elevated levels of organic compounds in the intake water (Figure 4.1). This approach incorporated a feed-back loop for the readjustment of the monitor/alarm to tune the system specifically to organics.



PHYSIOGRAPH CALIBRATION FLOW-CHART

If the feasibility study produced encouraging results, a second phase to develop a continuous monitoring system would be initiated interfacing the hard copy recording system to a computer. Otherwise, dose-response experiments with selected toxicants would be undertaken to ascertain the sensitivity of fish physiograph monitoring.

4.3 SYSTEMS DEVELOPMENT

4.3.1 General Systems Description and Key Components

A review of the 7 fish physiograph systems under development in the world was conducted. This review covers aspects considered in the development of the experimental system used in this study. A schematic diagram of each system is included in Figures 4.2 to 4.7, key components are described, considerations regarding test organism selection are covered and important points in test procedure and data analysis are reviewed.

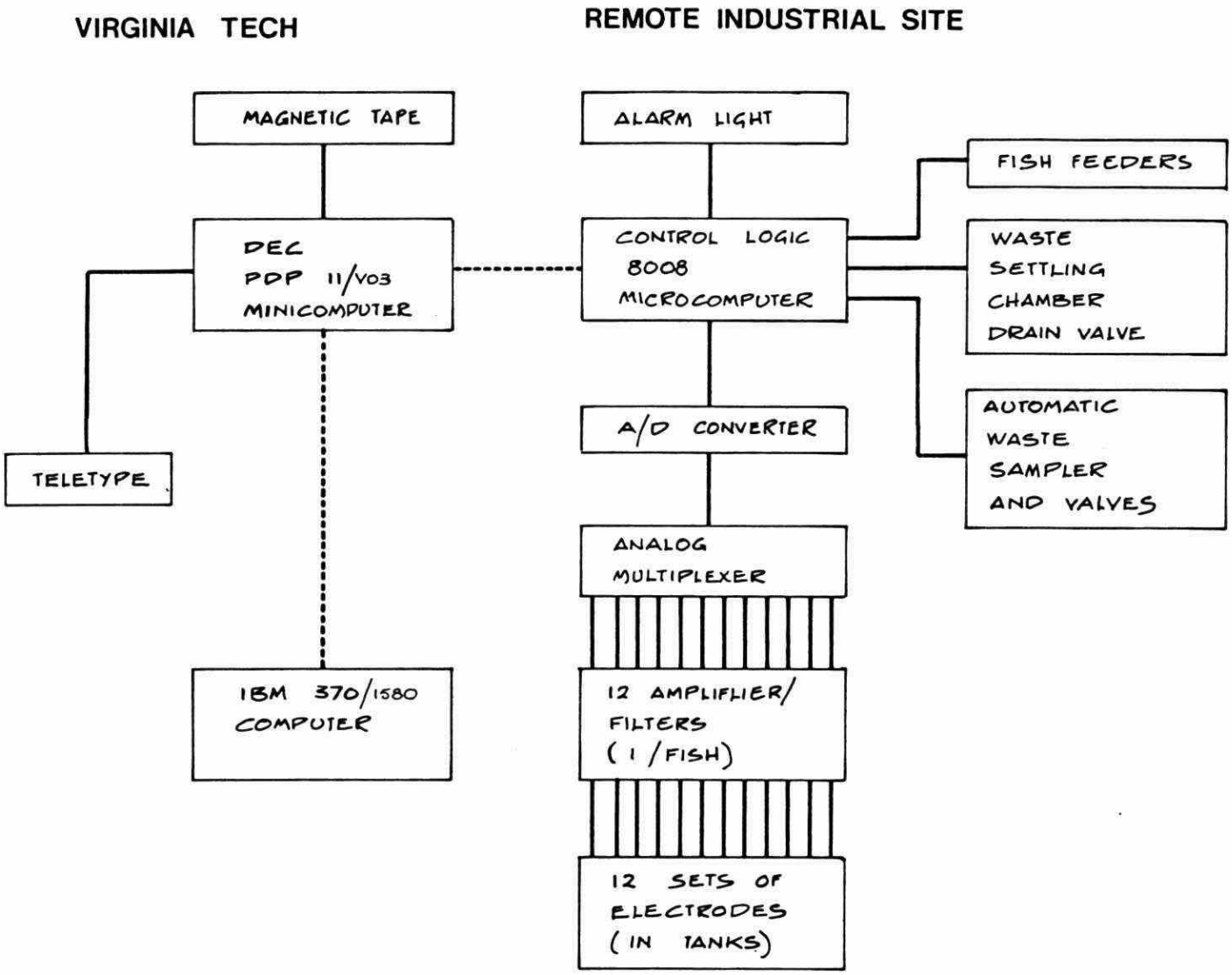
Simply, a fish physiograph system operates in the following manner: free-swimming fish are confined individually in monitoring cells. The microvolt bioelectric potentials emitted as they respire are received by two submerged hard wire electrodes affixed to the ends of the holding cells and are carried along shielded conductors to high gain frequency selective amplifiers and frequency filters for processing. At designated intervals, a mechanical switching device or programmed multiplexer pass the amplified signals to either a hard copy recording system for manual interpretation of the complete respiratory waves or to an A/D converter interfaced with a computer to compile and evaluate the respiratory data. If the fish activity deviates beyond certain critical limits, alarms are activated and remedial action may be taken to restore water quality. The following sections elaborate on this type of system and its operation.

Test fish

The respiratory rhythm of freshwater and saltwater fish representing more than 20 genera have been successfully recorded (15, 62, 63). A number of the species have been exposed to toxicants which produced alterations in respiratory pattern. The fish include several centrarchids such as largemouth bass, yellow perch, pumpkinseed and bluegill sunfish; some cyprinids, such as goldfish and fathead minnows and numerous salmonid species including pink, Atlantic and coho salmon, and both brook and rainbow trout. Yellow fish and Mossabmique bream, not endemic to Canadian waters, have been used for biomonitoring studies in Africa (64). Most studies have

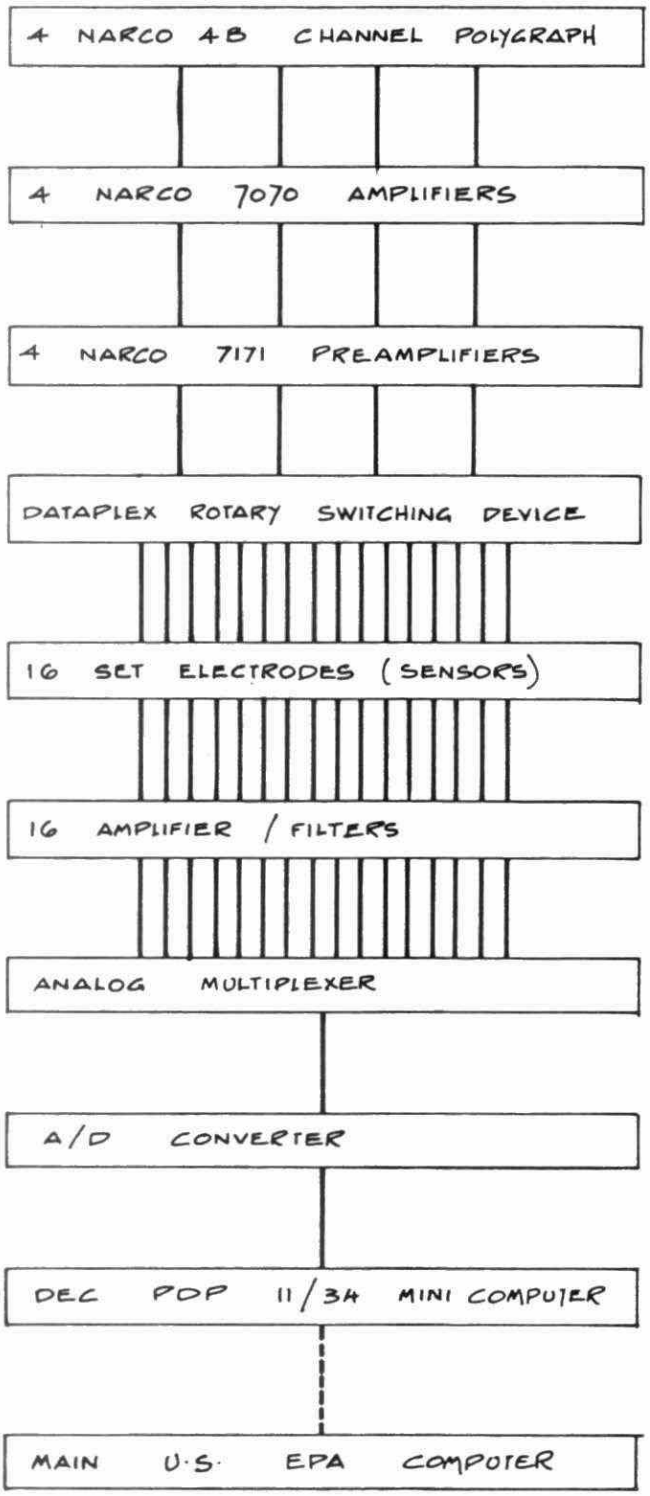
VPI & SU SYSTEM

4.2



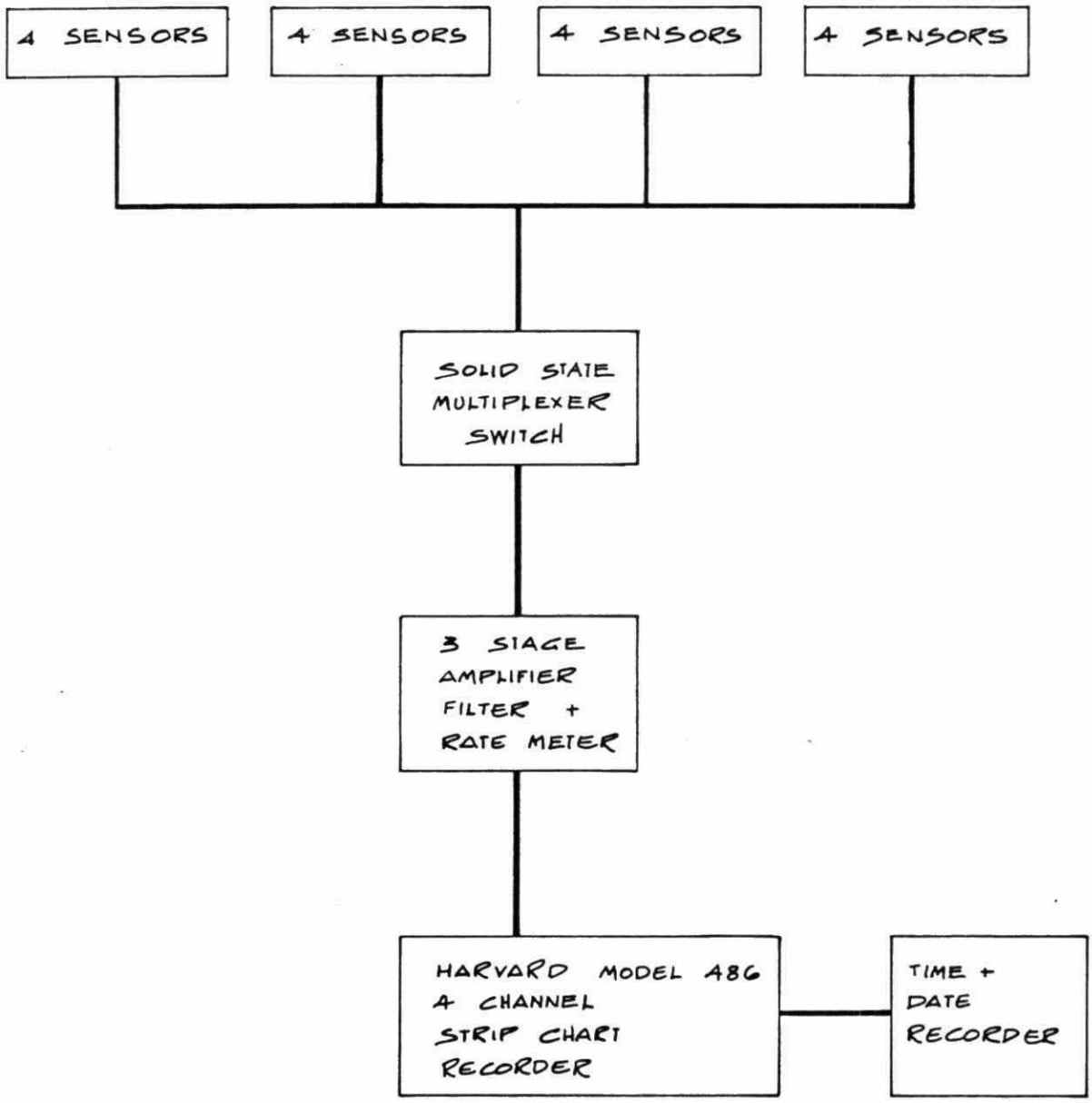
US EPA DULUTH SYSTEM

4.3



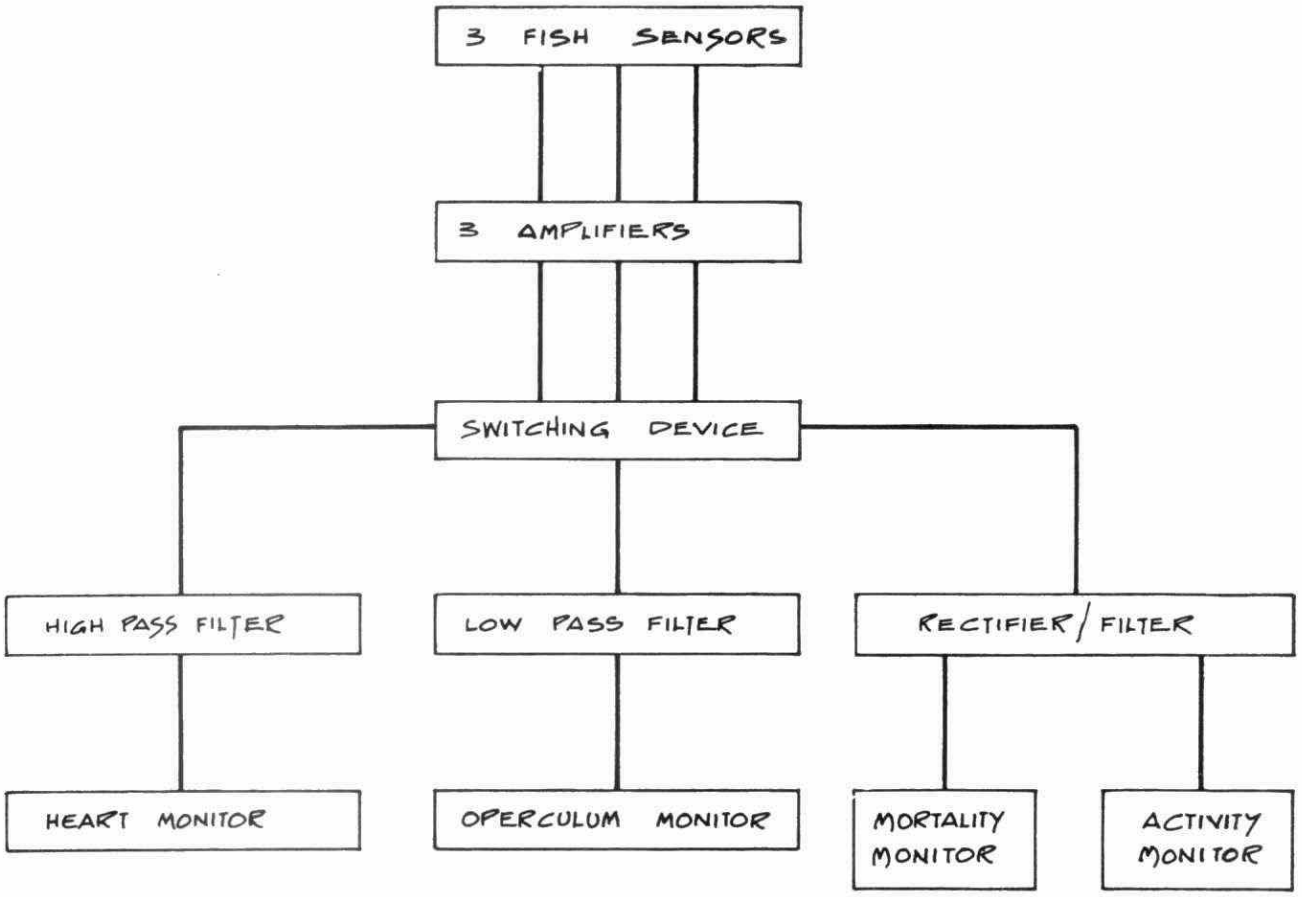
PROCTOR & GAMBLE SYSTEM

4.4



WRC SYSTEM

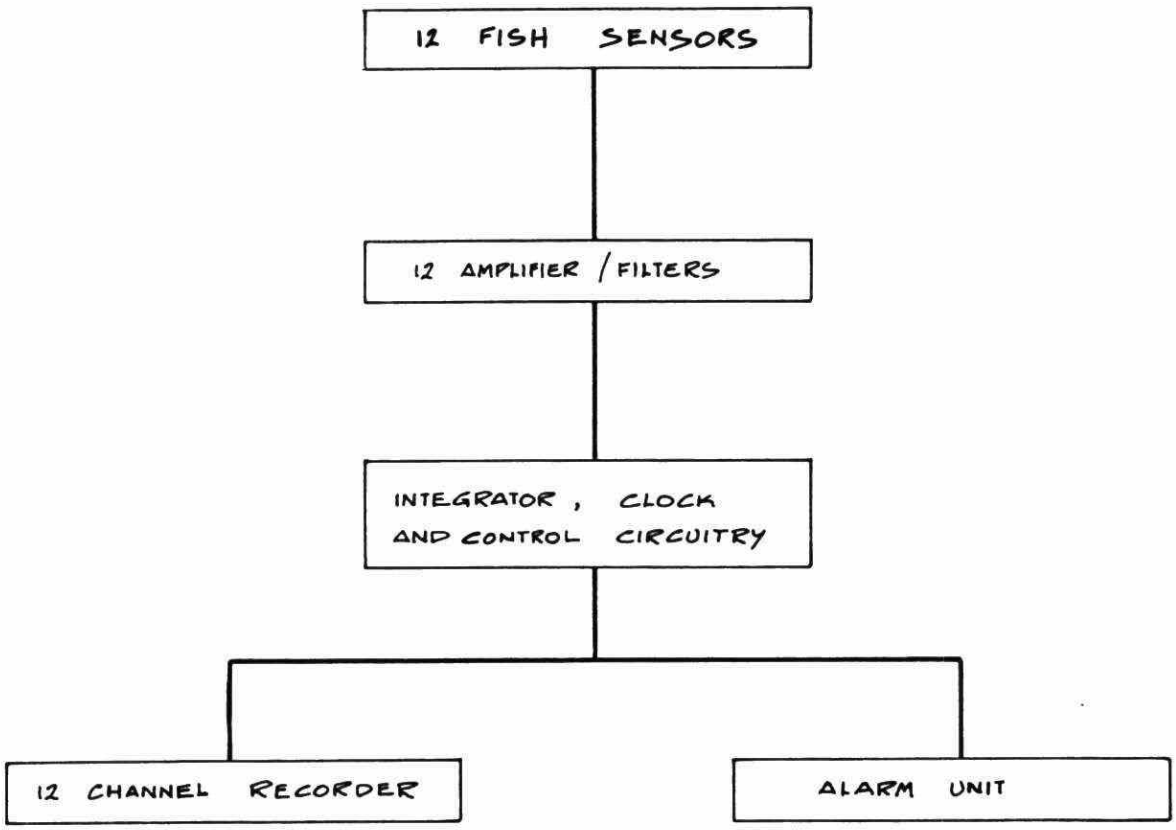
4.5



Computer automation under development

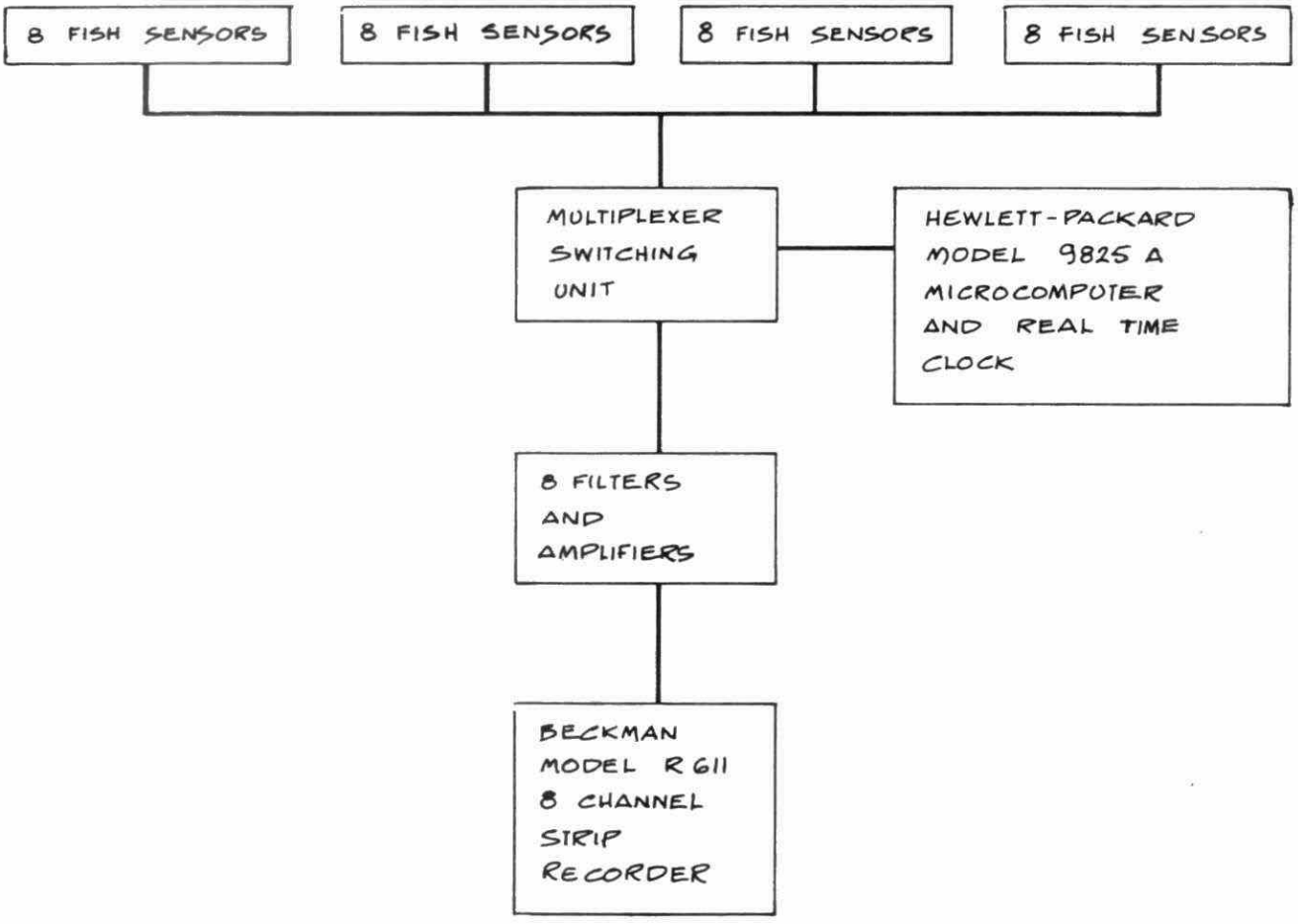
NIWR SYSTEM

4.6



US EPA CINCINNATI SYSTEM

4.7



NIWS SYSTEM

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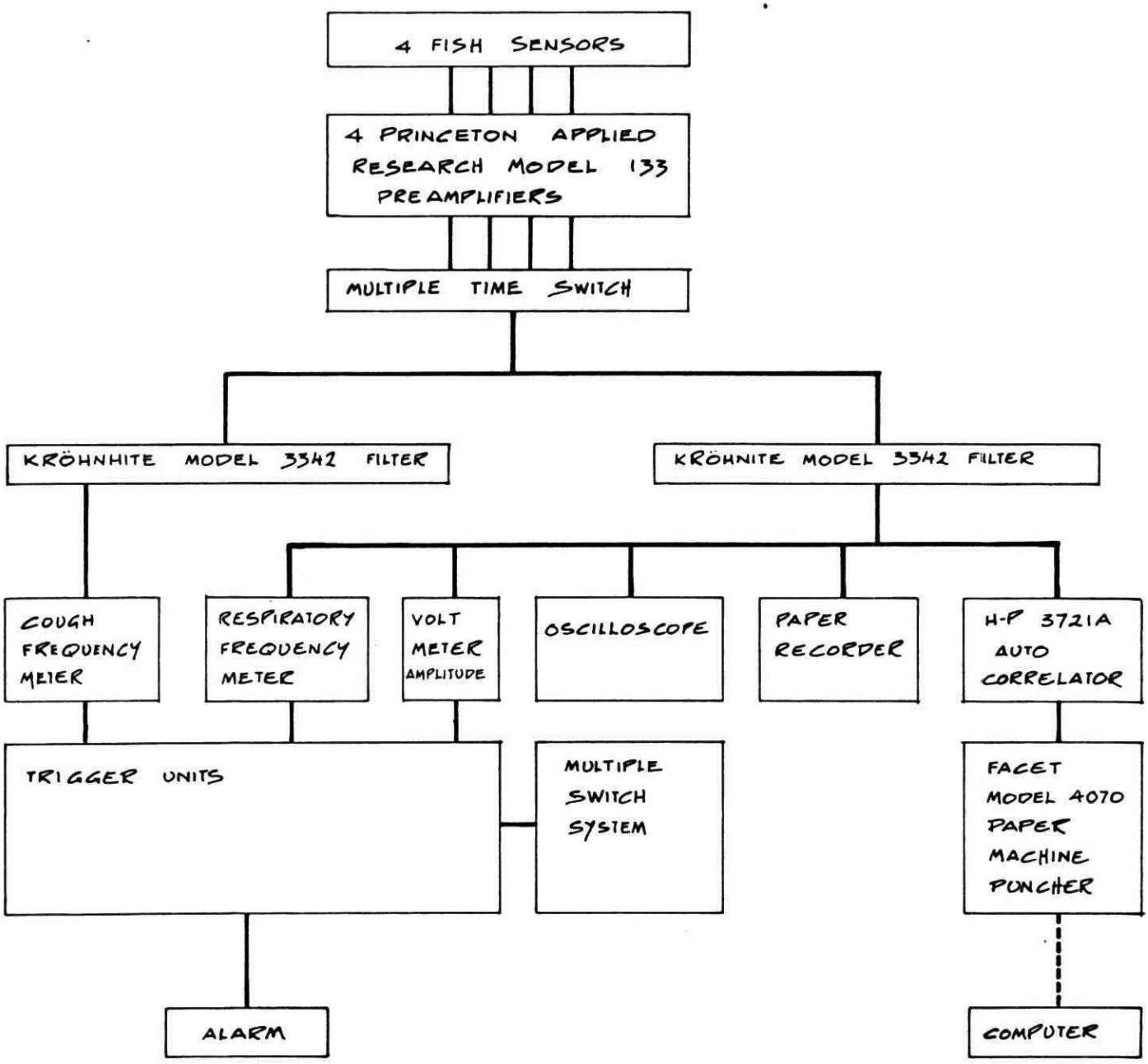


used bluegill sunfish, rainbow trout, brook trout and largemouth bass (33, 55, 59, 65). All are indigenous to North America and considerable toxicity data are available for each of these fish. However, no standard test fish has been developed for physiograph testing.

Bluegill sunfish and other centrarchids are favoured in the U.S. as they are quiescent in the electrode chamgers, readily available and have a broad temperature preferendum. Rainbow trout, on the other hand, exhibit greater spontaneous locomotory activity in the fish cells which interferes with interpretation of the respiratory recordings. In addition, they have more severe temperature requirements. However, rainbow trout have certain advantages such as a less variable ventilatory rate and a more uniform unimodal signal wave which may be easily computer-interfaced (63). Fish of different species exhibit different thresholds of respiratory response (15). Acute toxicity data suggest that rainbow trout is one of the more sensitive test species (66).

Number of test fish

Biomonitoring of lethally toxic solutions requires only 3 or 4 fish sensors. Although as few as 2 fish per treatment have been used, systems of this size are plagued with false detections or lack sensitivity (67). To detect lower concentrations of toxicants more experimental fish are needed to account for variability in response (68). As many as 8 fish in each group have been used to detect concentrations of KME, chlorinated hydrocarbons and TRC (49, 58, 61). However, 4 or 5 fish sensors in each group have been successfully used to detect sublethal concentrations of various surfactants, heavy metals and pesticides (46, 48, 57).

In essence, the greater the number of sensors used the more sensitive the system. A large number of fish allows for unresponsive fish and also gives latitude in the definition of an alarm condition so that the frequency of false detections can be minimized.

Size of fish

When selecting the size of the test fish a compromise must be made between the strength of the respiratory signal desired and the resistance of the fish to toxic slugs. If the fish are too small, they do not emit a respiratory signal of sufficient intensity to amplify independent of noise and body movements. Also, large fish may be less responsive to poisons than small fish.

Although fish as small as 0.4 g and as large as 120 g have been used in fish physiograph systems, young fish of approximately 5 cm in length and several grams in weight appear to best meet the requirements of these systems (35, 48, 57, 58, 69). Fish smaller than 50 mm in length are less suitable due to the small ratio of respiratory signal strength compared to electrical noise and body movement signals which make respiratory signal isolation difficult (63).

Alternate test animals

Spoor and Dawson of the U.S. Environmental Protection Agency have developed a method to monitor the total activity of daphnids (68). Strong signals are picked up when the animal is kept within 1 cm of the electrodes. Crayfish pleopod movement is another activity which can be automonitored (70). Recently Coyer demonstrated potential for using Cancer spp. heart and gill rhythms in biomonitoring (71).

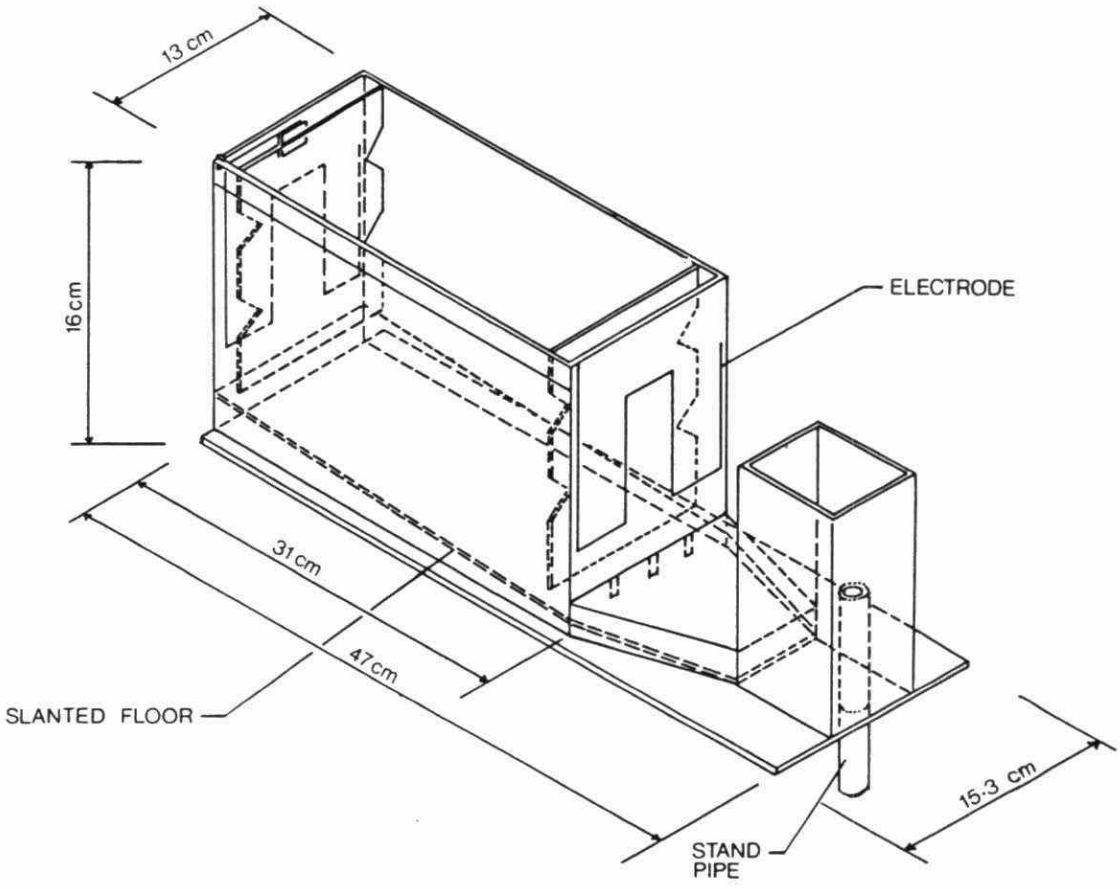
Electrode chambers

The chambers used to hold the test fish may be constructed either of glass or plexiglass. Various designs have been used in the past but the concept is the same. The chambers are rectangular in shape and sufficiently confining to maintain the fish in an orientation to the electrode plane and to minimize fish movement in the enclosure. A 5 L. holding capacity is adequate. Two variations of fish enclosures are illustrated in Figure 4.9.

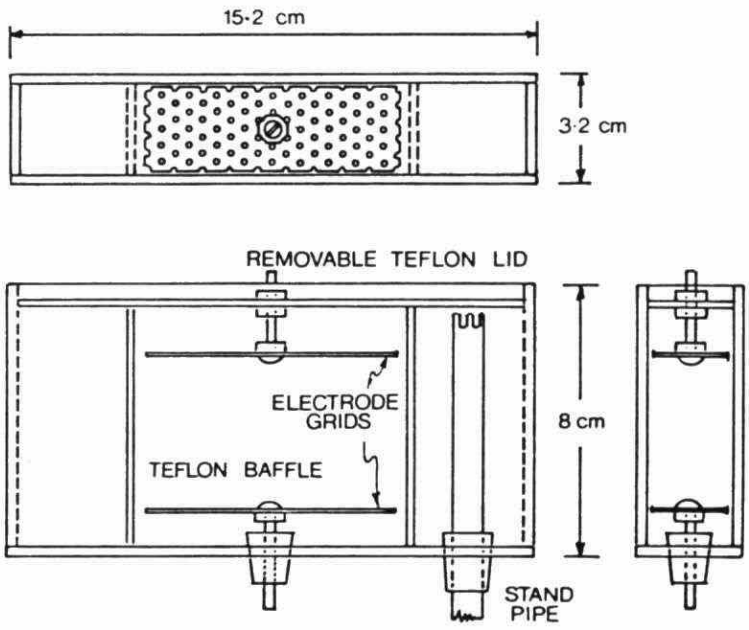
Typical Electrode Chambers

4.9

Gruber et al, 1980
60



Capute, 1980
54



If solids accumulation is anticipated, the electrode chamber should be modelled to be self-cleaning by slanting the floor of the cell and adapting bottom draw overflows at the effluent end of the chamber. Water flow of between 0.5 to 1 L/min. minimizes the accumulation of settleable solids.

Electrodes

Plate, wire or mesh electrodes constructed of an innocuous, conductive material such as stainless steel grade 304 have been used with success. Wire electrodes may be of various configurations (eg. U shaped) although straight wires are effective (48).

The electrodes may be positioned in front and in rear, or above and below the fish (32, 60). In either orientation, the signal strength increases the nearer the electrodes are to the fish. When placed anteriorly and posteriorly, signal strength is effected by the orientation of the fish and is greatest when the long axis of the fish is perpendicular to the plane of the electrodes. Also, when positioned in this manner the signal amplitude decreases and signal polarity reverses as the fish turns around in the enclosure. Electrodes situated above and below the fish minimize the influence of fish movement on signal amplitude and polarity is not affected.

Signal carrier cables

The bioelectric signals from small fish received by the electrodes range in strength from 0.01 and 40 uV but usually exceed 5-10 uV (72). Signal carrier cables used to transmit the signals are connected to the electrodes above the surface of the water in the fish cell, to eliminate excessive electrical interference. Coaxial cables shield the signals from noise and electro-magnetic interference from other electrical sources. This is particularly important between the electrode cell and the input of the amplifier due to the weakness of the unamplified signals.

Amplifier/filters

Various commercial (e.g. Grass, Princeton, Dymec, Beckman) and custom-built units are available to amplify the micro-volt respiratory signals of fish independent of electrical noise. They all have the essential characteristics of high impedance input, high gain amplification and active low pass filtering.

The amplifiers operate at a gain of approximately 10^4 to 10^5 while the filters have low and high frequency cut-off limits of 0.5 and 5-10 Hz. Above this range electrical line noise can interfere while body movements other than respiratory can pose difficulties at the low extreme. Band pass filtering at 20 to 40 Hz has been used to isolate fish heart recordings by WRC researchers (58).

Signal selection

Once the respiratory signals are amplified and filtered, they may be passed through a mechanical relay to a linear strip chart recorder for full inspection or to a multi-channeled analog multiplexer for computer analysis of any selected fish signal. Prior to entering the computer an A/D converter changes the analog respiratory signals to a recognizable numeric form.

Signal analysis

Manual interpretation of respiratory data allows detailed examination of the wave patterns but is too time consuming for real-time monitoring. The first methods of automatic signal analysis used voltmeters. In 1977, Morgan used a voltage integrator to assess the respiratory rate of the test fish (46). A similar arrangement of fixed threshold voltage counters was used to determine the respiratory rate of fish in the first computer-assisted monitoring system (73). However, problems of signal drift created inaccurate assessments and led to the development of a software method of measuring respiratory rate (74).

Basically, the program defined a respiratory cycle as consecutive, positive and negative pulses exceeding 1 volt in magnitude (75). The negative voltage surge beyond threshold clears the counter logic while the logic is clocked and a count registered by a following positive signal. An automatic gain control device alleviates the problems of signal variability over time and differences between individual fish that fixed threshold devices could not overcome.

Computers

The first published report of a computer applied to a fish physiograph monitoring system was in 1975 (73). In 1980, only 2 systems used computers to enable real-time toxicity monitoring (VPI and SU and US EPA, Duluth). These systems use DEC PDP11 minicomputers VPI uses model/V03 and EPA uses model /34.

Although these computers have the speed and storage capabilities to operate a biomonitoring facility independent of assistance; at present, larger computers are used in conjunction with these units to enable data assessment by several methods simultaneously. Factors such as assessment sample size, monitoring frequency, and number of fish are known to greatly influence the sensitivity and number of false detections of a system and research to assess these influences has not been completed. Once suitable monitoring procedures have been established, less costly computer packages assembled to perform this task will replace the less practical large computers.

4.3.2 Procedural Considerations

Acclimation period

From 6 to 12 hours to as long as 8 to 12 days has been used for the readjustment of the fish following transfer to small exposure chambers (32,37). Poels found that 2 to 3 days was not sufficient for complete recovery in his study and usually, 5 or 6 days acclimation is recommended (26, 32, 56). Cairns and Gruber use independent groups of fish and allowed at least 7 days recovery without any loss of monitoring time (73).

Control fish

Whenever possible, control fish receiving high quality diluent water or upstream receiving water should be used to indicate changes in basic water quality or to register extraneous influences such as physical distrubance. Vibrations, telephone ringing or sudden changes in lighting may cause significant changes in fish respiratory activity (30, 66).

Re-introduction of fish following alarm situations

Several new test fish may be added to the electrode chambers at one time to ensure continuous monitoring. Two banks of fish may be used where one group is monitored while the other is allowed to acclimate.

Adaptation of response

Replacement of the test fish at regular intervals (e.g. every 2 weeks or monthly) minimizes possible problems arising from loss of sensitivity (26, 88). There is some evidence to suggest that acclimation may occur following long term exposure to low levels of toxicants. Bluegills showed some decrease in activity responses when exposed to 3.0 mg/L zinc following a 29 week pre-exposure to 0.075 mg/L zinc although 41 weeks pre-exposure did not result in any altering of ventilatory responses (89).

A tendency for cough rate to return to pre-exposure levels on continuous exposure to BKME has been reported (41, 63). The time of acclimation to sublethal concentrations of BKME was directly related to the effluent concentration. Similarly, a gradual subsidence in rate of opercular movement during continuous exposure to copper has been noted (71, 90).

Repeat exposure to carbon tetrachloride, chloroform or tetrachloroethylene after a 4 to 5 day recovery period has resulted in duplication of effects on signal amplitude (54). In another study, respiratory responses were repeated 8 times when exposing bluegills for 5 hours to the 96 h LC50 concentration of zinc and copper when each application was separated from the next by a 5 hour interval (59). These limited results suggest acclimation does not occur following short-term exposures. Although it is not advisable to re-expose fish as they are known to adapt to toxic conditions, US EPA frequently re-uses fish up to 6 and 7 times if they respond well and the tests are for effluent monitoring and not research (80).

Diurnal variation

When fish are exposed to a natural photoperiod, breathing rates vary throughout the day in a predictable diurnal pattern (49, 77). The day can be divided into four distinct activity periods: 1) a period of low level respiration during the night hours; 2) a period of rising respiration at dawn; 3) a period of high respiration commonly with two peaks of increased respiration, one at noon and the other towards dusk; and, 4) a period of rapidly decreasing respiration during night-fall (30, 64). This heterogeneity in respiratory rate may be damped by maintaining the fish under continuous daylight although subjection to dimmed lighting is recommended for long-term studies (70, 88). Diurnal variation in gill purge rate is insignificant (63).

Test-fish are generally not fed in short-term research studies (49, 51, 54, 56). Long- term tests require fish feeding and an automatic pelletized food dispenser (32, 73). Live food e.g. Tubifex worms or Tilapia has been used in some systems, but feeding is manual (38, 50). Feeding should take place at the same time each day and alarm systems should be desensitized for a half hour following feeding to prevent activation during this period of increased activity (72). It is noted that depressed or absence of feeding also indicate a sublethal toxic response.

After 7 days exposure to pesticides, largemouth bass did not take live Tilapia immediately, and in some cases some time passed before food capture (50). Similarly, the feeding of brook trout was markedly less aggressive or ceased after 2 hours exposure to 9 ug/L copper or higher and nearly 40% of the fish exposed to 6 ug/L copper did not feed (90).

Interpretation of recordings

A comprehensive cinematographic examination of bluegill sunfish and fathead minnow ventilatory waves was conducted by Gruber and Associates (91). They found the usual respiratory waveform followed a bimodal pattern which varied in frequency and shifted to unimadality in response to stress. Portions of the recorded waveforms corresponded to periods of mouth and opercular movements. Variation in ventilatory signal waveform are presented. Carlson recently completed an investigation of types of gill purge response of bluegill sunfish and their interpretation (75). Acclimated bluegills were found to normally display an arrhythmic respiration, in which apneic periods alternate with groups of ventilatory cycles. Carlson identified three types of bluegill coughing patterns displayed by well-rested fish.

Data compilation and evaluation

A standard method has not been adopted for monitoring and evaluating fish respiratory activity or defining alarm conditions. All approaches have varied in one aspect or another. The VPI & SU and the NIWR are the only systems which compile data automatically and analyze data on a real-time basis. Only the VPI system uses computer-assisted programs with any degree of versatility. The other systems are restricted to off-line manual analytical procedures.

Fish respiration is usually monitored every hour or second hour for 1, 2, 3, 5 or 15 minutes (32, 49, 54, 56, 70). In the case of the NIWR system, 1 minute rates are compared at 1 minute intervals to pre-determined, maximum respiratory rates for each of the 12 sensors (46). Due to the computer interfacing of the VPI & SU system, the respiratory rates of each fish sensor are usually determined at 1 to 15 minute intervals. However, all the data generated by each fish may be collected and analyzed. It has recently been suggested by Cairns that data monitoring of not less than 10 minutes should be used for each assesment due to the large variability in ventilatory behaviour (73).

To alleviate problems due to diurnal variation in respiration, data may be lumped into two time periods i.e. light and dark, 4 time periods or hourly periods (49, 56, 60). Respiratory rates derived for the fish during toxicant exposure may be compared with 95 or 99% confidence limits calculated prior to toxicant exposure, to delineate the bounds of normal activity (49, 50, 51, 54, 65, 71). These standardization periods are usually 3 to 7 days, although as little as 6 hours has been used (37, 46, 56, 60, 84). Acitivity which falls outside the limits of respiration during standardization periods is considered behaviour altered from normal.

Statistics to compare mean respiratory rates between control and exposure periods for each time interval and exposure include paired t-tests and Dunnett's procedure (51, 56). To compare groups of respiratory rates, two way Analysis of Variance has been used although $\log_{10}$ transformation of the data was necessary prior to using this package to stabilize the variances (56). Another test examined the heterogeneity of variances on the assumption that stressed fish exhibit a more variable respiratory rate than unaltered fish (30). The concept of applying some limits to represent the normal range of respiration for subsequent comparison with those calculated under conditions of exposure has been used by all researchers. Recently, comparison of respiratory rates for each fish with an average from several hours previous using a Time Series analysis (i.e. sliding analysis of variance) has been found to be more useful (60). The non-parametric

2-tailed Mann-Whitney "U" test used by WRC is a variation of this approach and compares recordings of any two hour period for each fish with those of the corresponding period 24 hours earlier but violates the statistical assumption of independence for this test (58).

The criterion to designate an alarm condition is important in defining a system. It is a trade-off between the number of false positives one wishes to permit and the lag-time preceding detection of an alarm condition. The more stringent the criterion, the fewer the false detections but the longer the lag-time. Several sensors must simultaneously exceed the normal limits of behaviour to activate an alarm. Researchers have used response of 50% to 75% of the fish sensors to denote a stress condition (15, 49, 70 89).

Computer-assisted automation is recommended to maximize the system capabilities. Simply the logic of microcomputers has been implemented to control the timing and sequencing of fish respiration monitoring (54). Furthermore, computerization would enable real-time and frequent assessments using statistical packages; a necessary condition if practical application of physiograph systems is realized. Several researchers presently using off-line manual analytical procedures are reported to be interfacing their systems with computers e.g. WRC, Proctor and Gamble, VPI & SU (78).

In early 1981, only the VPI & SU and US EPA Duluth systems have developed software suitable for the monitoring of fish ventilatory activity. US EPA Cincinnati was in the process of developing a program to distinguish gill purge from respiratory movement but progress had to be halted due to a shortage of funding (78). VPI & SU have developed a battery of 15 programs in Fortran (Assembler) language to run their PDP 11/V03 based system. Developed over a period of almost a decade, their programs monitor the ventilatory behaviour of each fish by sampling each signal approximately ten times per second. It monitors chemical and physical water parameters, generates data to an oscilloscope or strip chart recorder and triggers the alarm system.

Although this software is commercially available (approximately $5,000.00 U.S. in 1981) transfer to an identical system would entail expense and time (88). Undoubtedly, some hardware related instruction changes would be required as well as customized changes such as alarm activation. The US EPA Duluth system's software is less sophisticated but effective and uses confidence limits delineating normal behaviour to discriminate changes in ventilatory rate. The Duluth system also uses a PDP 11/34 minicomputer. The minicomputers used in both these systems are backed by larger computers (e.g. VPI & SU system, IBM 370/1580) to assist data evaluation.

The VPI & SU biomonitoring system cost U.S. $120,000.00 unassembled. The package includes the PDP 11/V03 minicomputer and software, a mobile laboratory and the electronics and electrode chambers for 24 fish sensors (88). This cost may be reduced if the user is already equipped with the appropriate computer hardware and wishes to use the system where a mobile laboratory is unnecessary. Less costly computer automated systems will be developed with advancing computer technology. Some microcomputers have sufficient speed and storage capabilities for continuous water monitoring. Evidence suggests that the use of fewer sensors monitored or less frequent assessments may result in reduced sensitivity of these systems (72)(73).

4.3.3 Selection of the Monitoring Systems

Initially IEC proposed to use a packaged hard copy fish monitoring system supplied by the Water Research Centre, Stevenage, England. However, following a detailed evaluation of physiograph alternatives, the VPI & SU system was selected as the system most likely to meet the objectives of the research project. The WRC system was not availabile due to patent concerns in the United Kingdom.

A summary evaluation of alternate biomonitoring systems considered by IEC is included in Table 4.5 which show the criteria on which the VPI & SU alternative was selected. The primary advantages of using the VPI & SU system were commercial availability, documented capabilities and incorporation of a hard copy system as well as computerized capability. The hard copy portion of the VPI & SU system was proposed for Phase I of this study and if the feasiblity of drinking water monitoring was demonstrated, continuous monitoring capabilities could be achieved by adding on the computer interfacing.

TABLE 4.5: SUMMARY EVALUATION OF BIOMONITORING APPROACHES

	CENTRE FOR ENVIRONMENTAL STUDIES VPI & SU	SOUTH AFRICAN WATER RESEARCH NIWR	WATER RESEARCH CENTRE STEVENAGE WRC	HEWLETT-PACKARD	MODIFIED MOE
SYSTEM COMPONENTS					
Exposure Equipment	Exposure cells and amplifier/filters.	Exposure cells and amplifier/filters.	Exposure cells and amplifier/filters.	Exposure cells and amplifier/filters.	Exposure cells, amplifier/filters and multi-channel switcher.
Data Acquisition Equipment	Software based mini-computer controlling alarm circuits.	Hardware based integrator and alarm circuits integrated with amplifier/filter with digital output.	Hardware based monitors and alarm boxes integrated with amplifier/filters with strip chart output.	Software based micro-computer controlling alarm circuits.	4-channel strip chart with no alarm circuitry.
ACTIVITIES MONITORED	Ventilatory rate and amplitude.	**Ventilatory rate as beats/30 sec.	*Cardiac, ventilatory and total activities.	Ventilatory rate and amplitude.	Ventilatory rate and amplitude.
NUMBER OF FISH SENSORS	12	** 5	** 1-3	12	4-12
CAPABILITIES	*State-of-the-art monitoring system. Industrial effluent field monitoring proven; has capabilities as yet undeveloped.	Used as a short-term sublethal laboratory bioassay system; has been developed to its limits.	Limited technical information, automation under development.	**Undeveloped, may be adapted to CES system with limited processing speed.	Used for short term effluent monitoring and sublethal bioassays.
DATA ACQUISITION AND ANALYSIS	*Computerized, High speed, Capable of analyzing full ventilatory signal. *Software compensates for diurnal and feeding changes.	Hardware based fixed threshold alarms. Analysis on respiratory beats per 30 seconds.	Hardware based fixed threshold alarms. Monitors 3 activities of 1 fish or 1 activity of 3 fish (operator choice).	Computerized. Limited speed.	*Visual interpretation of strip charts. Manpower intensive.
DEVELOPMENTAL POTENTIAL					
Phase I (a)	*Very good	Very good	*Very good	Very good	*Very good
Phase II (b)	*Very good	Fair	Fair	Poor	Poor
FUTURE MONITORING APPLICATION	*Automated water or effluent monitor for regulatory or industrial applications.	Semi-automated effluent monitor for regulatory or industrial applications.	Needs automation for regulatory or industrial application.	Application limited by low data acquisition and processing speed.	Not capable of for long-term monitoring.
AVAILABILITY AND DELIVERY	Computer = 3-4 mos. Exposure cells and amplifier/filters = 3 mos.	Data compilor and processor = 5 mos. Exposure cells = 3 mos.	**Not available	Computer = 3 mos. Exposure cells and amplifier/filters = 3 mos.	Recorder = 1 wk. Exposure cells, amplifier/filters and switchers = 3 mos.
SET-UP TIME	Moderate	Short	Short	Long	Short
COSTS					
Equipment	$25,500	5-channel = $21,000 12-channel = $37,000±	$7,000; rental originally proposed no longer available.	$30,000	$8,000
Development & Service	$10,000	$ 1,000	0	$ 5,000 - 10,000	$1,000
Total	**$35,500	*5-channel = $22,000 **12-channel = $38,000	$7,000	**$35,000 - 40,000	*$9,000

Legend - * Pros
** Cons
(a) Phase I is the feasibility study to assess the ability to detect elevated organics by changes in fish activity.
(b) Phase II is the monitoring study which interfaces the physiograph with an automated (computerized) data acquisition and analysis system to develop an automated continuous monitor/alarm of elevated organics.

4.3.4 Development of Respiration Monitoring Systems

Respiratory signals emitted by free-swimming fish can be sensed by electrodes immersed in water. However, due to the weak signal, precise techniques must be used to isolate the signals which are amplified to levels independent of interferences. The following section outlines some difficulties encountered and overcome by IEC in the development of a reliable system to monitor and record fish respiratory signals.

A standard 4 channel polygraph equipped with low level preamplifiers was used in the initial attempt to record fish respiratory activity. Shielded cable joined to the polygraph at the preamplifer inputs was connected to a pair of wire electrodes immersed in the fish chamber to receive and transmit the signals. Variations in potential due to fish respiration, although successfully recorded with this system, could only be made at the highest preamplifier sensitivity (ie. 1-2 uV/division) due to its low amplification capability. Also, clear records were only made when aluminum screening was placed around the fish chamber as a Faraday cage to shield the electrodes from interfering signals. Grounding of all components of the system at a common point was required to prevent ground loop effects.

Drifting of the signal baseline was also a frequent problem. This resulted because the polygraph preamplifier was not designed to selectively isolate the respiratory frequencies from other body movement signals. This D.C. drift was controlled by attaching a 68 ohm resistor across the leads of the electrodes.

Signal loss due to moisture dissipation (ie. short circuiting), as well as static charge build-up hampered continuous monitoring. Momentary shorting of the electrodes usually returned the signal but was not a permanent solution.

Despite these difficulties, recordings of the ventilatory pattern of a number of fish species including goldfish, rainbow trout, brook trout, rock bass and largemouth bass were obtained. Visual observations ensured that the polygraph traces correlated with fish respiratory movements.

Subsequently, custom-built electrode chambers and noise-immune amplifier-filters designed for fish respiratory monitoring were acquired through Drs. John Cairns, Jr. and David Gruber of VPI & SU. The system enabled continuous recording of the signal. The amplifier had a high gain of 10^6 so that recording at 200 mV/division was possible making the signal more stable. The filter was designed to eliminate electrical noise and body movement other than fish respiration with selective frequency cutoffs at 0.5 and 8 Hz. Once wired into the system between the electrode chamber and the polygraph, loss of respiratory signals was overcome and due to the strength of the signal, the Faraday cage became unnecessary and was removed. Following adjustment of two variable resistors to align DC offset and set the desired signal level, the amplifiers operated continuously and except for replacement of one faulty capacitor, operated free of maintenance.

After this testing and adjustment period, a continuous flow system was set-up using the VPI & SU electrode chambers. The effluent tubes of the cells were enlarged to accept a greater water flow to minimize solids accumulation. A sound proof plywood enclosure with 10.2 cm insulated walls was constructed to house the exposure chambers. It was built on boxes filled with sand to reduce floor reverberation. Inspite of the installation of an air conditioner and two refrigeration units, the water temperature could not be lowered sufficiently to maintain trout in the facility during the summer months and largemouth bass were used in this final testing stage.

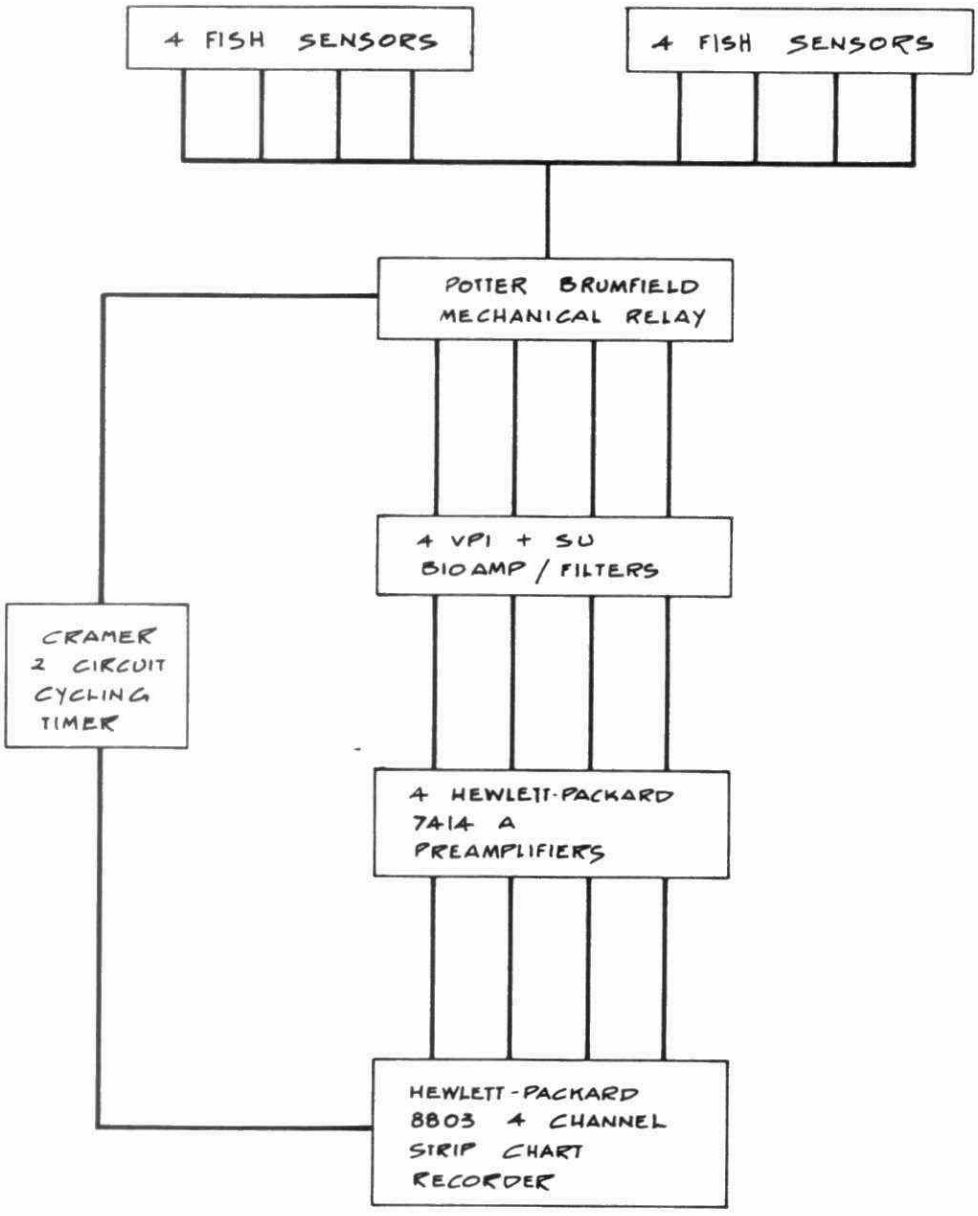
4.3.5 The Experimental System

The final hard copy recording system developed for this study is illustrated in Figures 4.10 and 4.11. It consisted of 8 electrode chambers designed by VPI & SU. Each chamber held one free-swimming fish (Figure 4.12 - Electrode Chamber). The chambers were contained in a vibration and sound proof enclosure equipped with artificial lighting, simulated photoperiod and tubes to feed the fish (Figure 4.13). A stainless steel electrode was attached with silicone sealant to each of the two end plates of the electrode chamber for signal reception. Mueller alligator clips soldered to the two conductors of the Belden shielded cable were attached to the wire electrodes.

The electrode signal was transmitted along the shielded cable through an electro-magnetic double-throw relay controlled by a continuous cycling two circuit timer to the amplifier/filters designed and built by VPI & SU. The Potter Brumfield relay and Cramer timer (Figure 4.12 - Switching Mechanism) enabled the monitoring of eight fish on the four channel strip chart by selecting the fish by banks of four. Ideally, switching of the biosignals should follow signal amplification but in this case only four amplifier/filters were available. Following processing, the signals were transmitted to a 4-channel Hewlett-Packard 7414A polygraph equipped with 8803A preamplifiers and recorded (Figure 4.12 - Biosignal Processing System).

Experimental System

4.11



4.4 MATERIALS AND METHODS

4.4.1 Study Area and Laboratory Facility

The downstream experimental site for the bioaccumulation study was also used to evaluate the fish physiograph monitoring system. Located at the Brantford Water Treatment Plant (WTP), the laboratory facility monitored the incoming water to the City of Brantford WTP. It was speculated that undetected slugs of organic compounds could enter the treatment facility as several urban industrialized communities upstream represented potential sources of organic compounds. A few taste and odour complaints had been reported in Brantford in the past.

The biomonitoring facility was located close to the raw water intake from the canal. The test laboratory was contained in a 4.6 m x 3.1 m room within the WTP. The laboratory water was drawn directly from the canal with no retention or dilution to assure detection of potential short-term changes in water quality. The power supply, transformer, pumps, heaters and coolers for the laboratory were located directly below the facility in the basement. Automatic in-line monitors provided hourly measurements of water temperature, dissolved oxygen, pH, turbidity and conductivity. The monitors/alarms described in the bioaccumulation experiment were also used in this portion of the study (see Section 3.3.3). The laboratory was distant from large motors, switches, selenoids and other electrical devices which could interfere with the physiograph uV signal amplification and recording equipment.

4.4.2 Experiment 1: Acclimation and Preliminary Response

In the first experiment, the respiratory activity of largemouth bass was recorded for a one week period while exposing the fish to the intake water. The experiment was to test the hard copy recording system for reliability under continuous use, to examine variation in fish respiration in a practical situation, to determine the time needed for fish to stabilize activity following transfer to the exposure chambers, and to assess the effect of a controlled dose of chloroform on fish respiration.

Largemouth bass (weight of 17 ± 3g) were transferred to four electrode chambers on 27 September 1980 following three weeks acclimation to the 20°C incoming river water. Respiration of each fish was then monitored for five consecutive minutes at two hour intervals throughout the seven day exposure. This monitoring was considered to be sufficiently frequent based on the response times of fish to organic compounds (Table 4.1) and the five minute monitoring compared with data collected by other strip chart recording systems (see Section 4.3.2 - Data Compilation and Evaluation). The physiograph was operated at a chart speed of 10 mm/sec and biopotentials were recorded at a sensitivity of 500 mV/division.

Dissolved oxygen, pH, temperature, conductivity and turbidity of the water was recorded on punch tape of the robot water monitoring unit every hour during the same 5 minute interval as the physiograph recordings. Calibration of physico-chemical monitors was checked daily. An artificial 9h day/15h night photoperiod was maintained during acclimation similar to the period used for the experimental exposure. Half hour light dimming and brightening occurred at 1700h and 0700h, respectively simulating dusk and dawn periods. The experimental fish were fed live meal worms ad libitum similar to the acclimation period. Cell flow rate of 600 ml/minute ensured a 95% molecular replacement time of less than 1/2 hour.

On exposure day 5, dosage of one of the fish with 3 mg/L of chloroform was initiated and continued for the remaining 40 hours of the exposure. A mariotte bottle was used to dispense a concentrated chloroform solution to the water stream entering the fish cell to maintain a constant exposure concentration. Control and experimental water samples were analyzed for chloroform and several other halogenated aliphatic compounds at MOE laboratories.

The strip chart recordings were analyzed manually for ventilatory and cough rate and amplitude of ventilation for each of the five one minute intervals monitored every two hours. The mean respiratory rate for each fish and its standard deviation were then plotted against time and compared to the water quality data. This allowed the determination of the time to recovery and the type of response elicited by chloroform dosing.

4.4.3 Experiment 2: Feasibility of Monitoring Variations in Quality of a Drinking Water Supply

In the first experiment, the reliable production of clean, stable signals proved the physiograph amenable to computerization for real-time monitoring. However, before interfacing the physiograph to a computer, its ability to respond to elevated levels of organics had to be demonstrated. Manual data interpretation to determine changes in signals associated with the response of fish to changes in water quality was required prior to automating the monitoring system.

Based on the absence of any significant changes in fish activity or water quality in the first experiment and to maximize data obtained on the physiograph's capabilities, the second experiment incorporated dose-response trials and monitored the raw water. These dose-response experiments using several compounds possibly present in the raw water at Brantford assessed the system's ability to respond to elevated levels of substances in the raw water. Control cells exposed to raw water served as monitors of raw water and also as references for the dose-response cells to confirm stress detection. Simultaneous water sampling and analysis were conducted for compounds in the control and dose-response cells.

Following discussions with MOE three compounds were selected for testing. The compounds selected were phenol, ammonia and zinc. All these compounds may occur in municipal and industrial discharges and may pose health hazards and create taste and odour. For example phenol contamination of a U.S. drinking water supply due to a spill had recently been reported (92).

The exposure test, including a six day acclimation period, lasted 22 days and was completed on 24 December 1980. As indicated in Table 4.6, two fish were exposed to each of the three compounds and two fish served as references. The compound concentrations were selected to encompass reported

TABLE 4.6 EXPERIMENT II EXPOSURE AND DOSING SCHEDULE

Date	Phenol		Total Ammonia		Zinc		Reference		
(Dec. 1980)	Period	Dosing (Fish 1 & 6)	Period	Dosing (Fish 2 & 3)	Period	Dosing (Fish 4 & 5)	Period	Dosing (Fish 7)	Dosing (Fish 8)
2	Acclimation	RW[1)]	Acclimation	RW	Acclimation	RW	Acclimation	RW	RW
3	Acclimation	RW	Acclimation	RW	Acclimation	RW	Acclimation	RW	RW
4	Acclimation	RW	Acclimation	RW	Acclimation	RW	Acclimation	RW	RW
5	Acclimation	RW	Acclimation	RW	Acclimation	RW	Acclimation	RW	RW
6	Acclimation	RW	Acclimation	RW	Acclimation	RW	Acclimation	RW	RW
7	Acclimation	RW	Acclimation	RW	Acclimation	RW	Acclimation	RW	RW
8	Baseline	RW	Baseline	RW	Baseline	RW	Baseline	RW	RW
9	Baseline	RW	Baseline	RW	Baseline	RW	Baseline	RW	RW
10	Baseline	RW	Baseline	RW	Baseline	RW	Baseline	RW	RW
11	Baseline	RW	Baseline	RW	Baseline	RW	Baseline	RW	RW
12	Initial Dosing	0.2[2)]	Initial Dosing	1.0	Initial Dosing	0.5	Reference	RW	RW
13	Initial Dosing	0.2	Initial Dosing	1.0	Initial Dosing	0.5	Reference	RW	RW
14	Initial Dosing	0.2	Initial Dosing	1.0	Initial Dosing	0.5	Reference	RW	RW
15	Initial Dosing	0.2	Initial Dosing	1.0	Initial Dosing	0.5	Reference	RW	RW
16	Initial Dosing	0.4	Initial Dosing	2.0	Initial Dosing	1.5	Reference	RW	RW
17	Initial Dosing	0.4	Initial Dosing	2.0	Initial Dosing	1.5	Reference	RW	RW
18	Initial Dosing	1.5	Initial Dosing	3.0	Initial Dosing	4.0	Reference	RW	RW
19	Initial Dosing	1.5	Initial Dosing	4.0	Initial Dosing	4.0	Reference	RW	RW
20	Initial Dosing	4.5	Initial Dosing	7.0	Wash Out	RW	Reference	RW	RW
21	Initial Dosing	8.0	Initial Dosing	10	Wash Out	RW	Reference	RW	RW
22	Lethal Dosing	20	Washout	RW	Repeat Dosing	2.0	Reference	RW	RW
23	-		Repeat Dosing	20	Repeat Dosing	4.0	Dosing & Reference	10[3)] 10	RW RW
24	-		Lethal Dosing	60	Lethal Dosing	10	Dosing	10	60[3)]

Note: 1) Raw River Water
2) Nominal Concentration mg/L^{-1}
3) Phenol Dosage

values known to elicit a respiratory response. The concentrations were increased sequentially simulating a chemical spill upstream. Moreover, this approach ensured the fish had not been previously exposed to a high concentration rendering them insensitive to subsequent exposures. The exposure to each concentration was usually 48 hours. When a response was observed, a washout recovery period was used to determine if activity returned to baseline. The recovery was followed by reexposure to examine if the response could be repeated. On the last two days of exposure, the two reference fish were spiked to provide additional data on sensitivity and respiratory response.

These experiments used largemouth bass (weight of 4.4 + .5 g) obtained from R. Goossens Trout Farm and held for three weeks prior to introduction into the electrode chambers. During this period they were fed neon tetras and gradually acclimated to the experimental temperature of 16°C. Largemouth bass were chosen rather than a cold water species as water temperature could be controlled by heating therefore avoiding an additional experimental variable.

On 2 December 1980, the fish were placed in the eight electrode chambers for a six day period of stabilization. Previous experiments had shown a four day acclimation period to be adequate. The acclimation was followed by a four day period to establish baseline activity. The respiratory activity of each fish was monitored at 2 hour intervals during the baseline period and the subsequent 12 days of dose-response. The physiograph was operated at a chart speed of 10 mm/sec and respiratory signals were recorded at a sensitivity of 200 mV/division.

A dosing system of mariotte bottles and mixing cells was used to introduce and mix the toxicants. A constant flow rate of 750 ml/min was delivered to each of the electrode chambers for the 22 day experiment. The 18L mariotte stock solutions were prepared by dissolving analytical grade crystals in known volumes of raw river water using a magnetic stirrer. Phenol solutions were prepared from pure crystals. Ammonia was added as ammonium chloride and zinc solutions were prepared using zinc sulphate.

Dissolved oxygen, temperature, pH, conductivity and turbidity were monitored automatically on punch tape at hourly intervals during the 22 day experiment. 20L composite samples of raw water were collected daily in clean, solvent-rinsed glass bottles for organic analysis. The samples were stored in an isolated area in the basement of the WTP at 4°C. The carboys were sealed with washed aluminum foil and stoppered. A subsample of each composite was collected for multi-element anion/cation scan.

Samples of the spiked water were collected at least daily along with similarly preserved samples from the reference cells to assess background levels of zinc, phenol and ammonia. The zinc, phenol and ammonia samples were analyzed at detection limits of 0.1, 0.0005 and 0.050 mg/L.

The physiograph strip chart was manually analyzed for respiratory rate, cough frequency and amplitude of ventilation at the end of the experiment. Respiratory rate for each fish was plotted and correlated with chemical dose. The sensitivity of the polygraph system to both natural and artificial levels of the compounds was then determined.

4.4.4 Experiment 3 The Effect of Zinc on Rainbow Trout Respiration

This dose-response experiment examined the respiratory response of rainbow trout to sublethal zinc exposure in water at 5°C. It assessed the feasibility of using rainbow trout as an alternate to warm-water species for monitoring cold water situations. Preliminary observations indicated that largemouth bass were less active in cold-water and reduced their ventilatory rate to low levels of less than 10 cycles/min at 5°C.

The methods used were similar to those in Experiments I and II. Four days stabilizaiton was provided. This was followed by one day baseline recording of the respiration of one control and one rainbow trout of 6.4 and 7.2 grams wet body weight respectively. A third electrode chamber held a 0.4 gram rainbow trout selected to examine the feasibiliby of recording respiratory activity of young fish to take advantage of their sensitivity.

The dose-response exposure was 36 hours. Zinc was added to the delivery water using a mariotte bottle. Water samples were collected and analyzed for zinc by pulse polarography at the University of Guelph.

4.5 RESULTS AND DISCUSSION

4.5.1 Experiment 1: Acclimation and Preliminary Response Evaluation

The VPI & SU amplifier/filters were highly reliable and produced clear, stable signals through the 7 day test. However, malfunction of one of the polygraph preamplifier choppers prevented collection of a full set of respiratory data for one fish.

Respiratory rate and amplitude of respiration varied considerably during the exposure and followed a diurnal pattern due to photoperiodism (Figures 4.14, 4.15, 4.16). However, coughs or gill purges were infrequent and showed no trend (Table 4.7). The water quality was stable during the experiment (Table 4.8) and precluded correlating fish activity to variations in water quality.

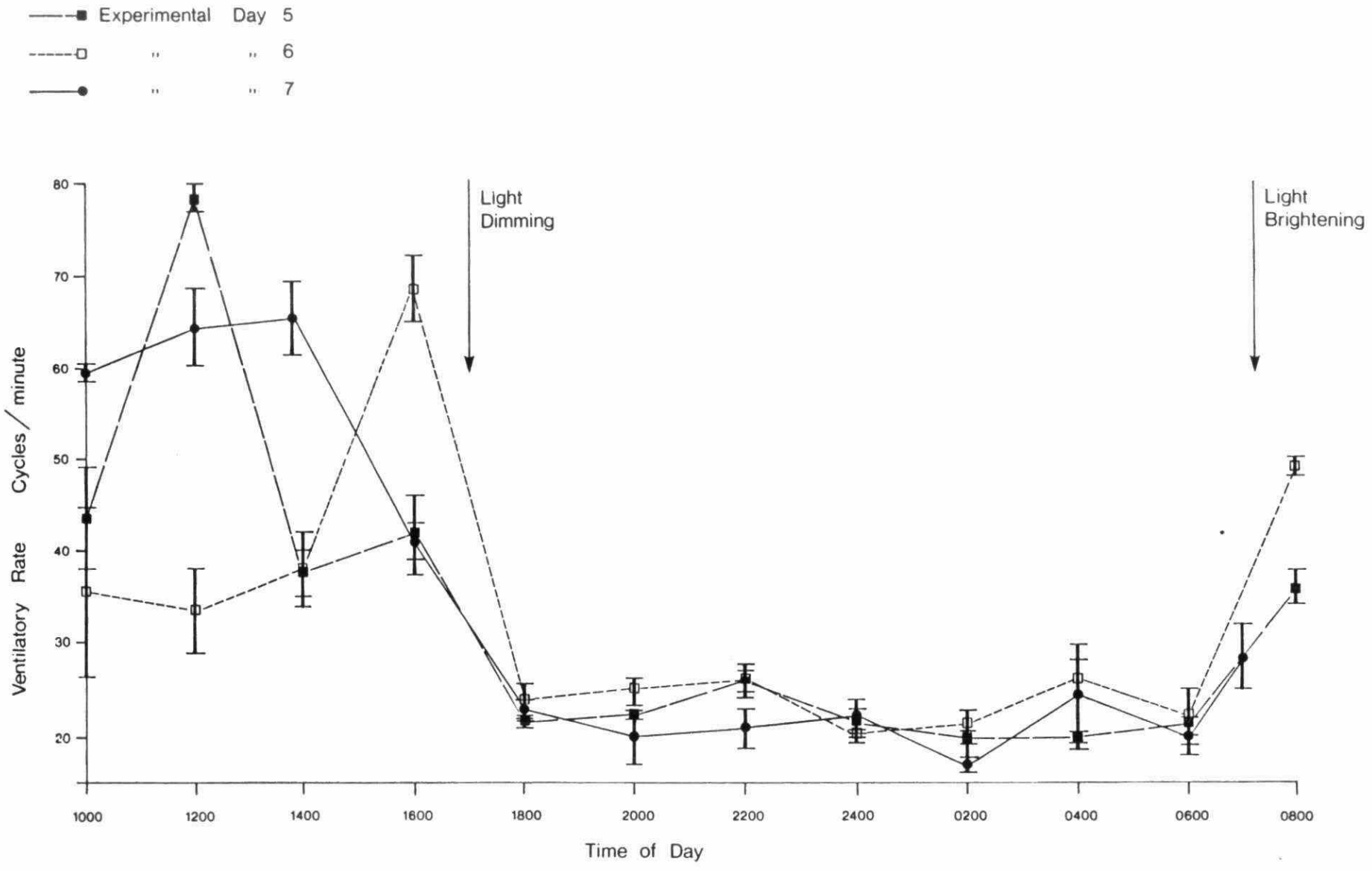
Abrupt changes in fish respiratory activity occurred following half hour light dimming and brightening (Table 4.9). Amplitude of ventilation and ventilatory rate was greater during the day than at night. Also, variation in respiratory rate and amplitude was lower during the night (Table 4.10). Previous researchers have made similar observations and have noticed more frequent responses during the dark interval (49, 89). Although many of these responses probably arose due to the statistical advantages afforded by lower respiratory rates and reduced variability, a circadian periodicity in sensitivity to toxicants may occur (89).

Based on the plots of fish respiration versus time, four days appeared adequate for fish recovery from the stress of transfer (Appendix 2). A half hour was necessary for the fish to return to baseline activity following feeding (Table 4.11).

Diurnal Variation in Respiratory Rate of an Acclimated Largemouth Bass

4.14

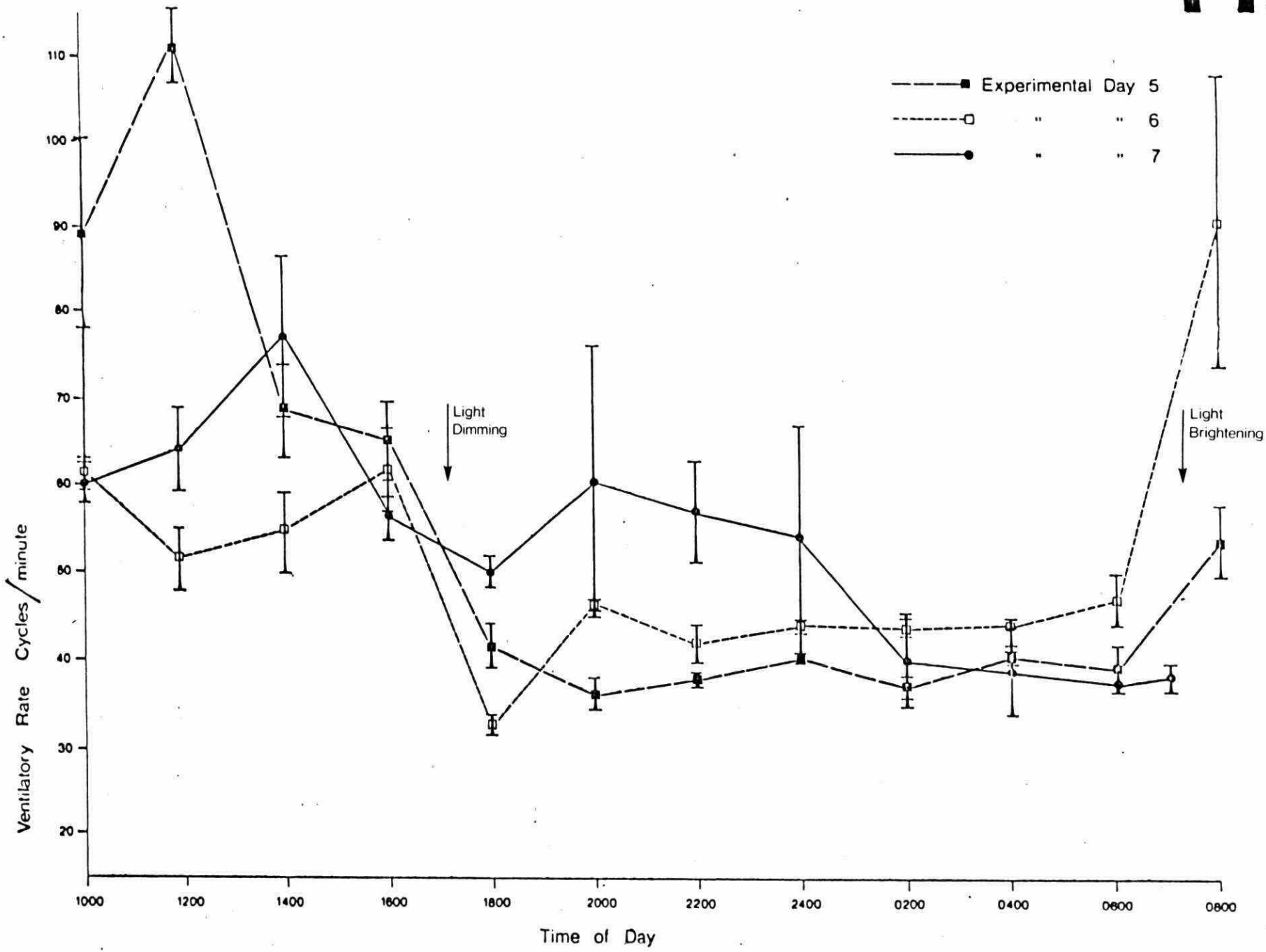
Experiment 1 Control Fish 1



Diurnal Variation in Respiratory Rate of an Acclimated Largemouth Bass

4.15

Experiment 1 Control Fish 2



Exposure of a Largemouth Bass to Chloroform

Experiment 1 Experimental Fish 1

4.16

Dosage with toxicant began Day 6 1600 hr.

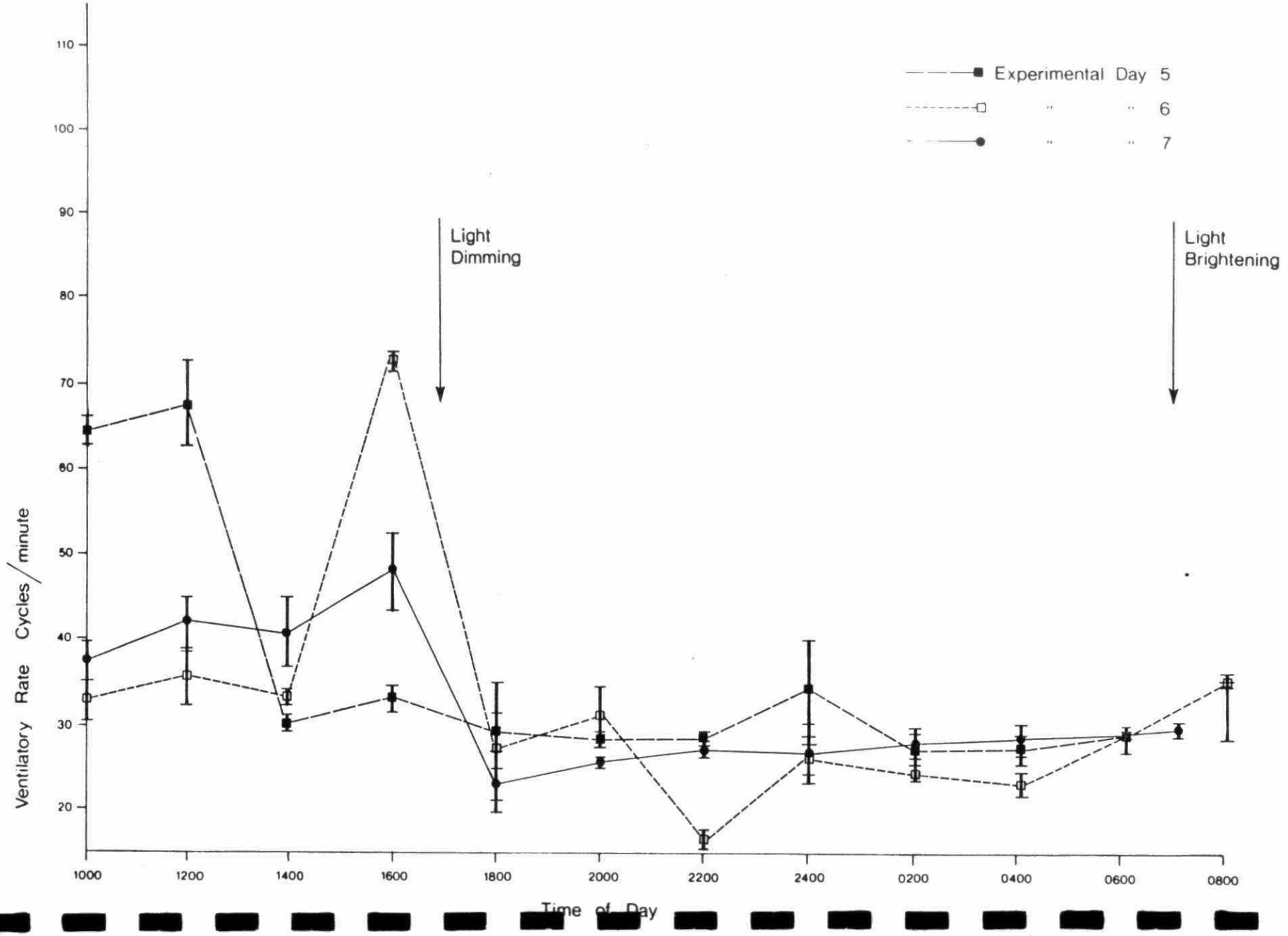


TABLE 4.7: EXPERIMENT 1 COUGH FREQUENCY (CYCLES/5 MINUTES)

Test fish	Test Day	Time of day											
		1000	1200	1400	1600	1800	2000	2200	0000	0200	0400	0600	0800
Control 1	1	0	0	0	0	0	4	1	0	3	0	2	0
	2	1	0	0	0	1	3	0	0	0	1	1	1
	3	2	0	0	0	0	0	0	0	2	0	2	2
	4	0	0	0	0	1	0	0	0	0	0	0	2
	5	0	0	0	0	1	1	0	0	0	3	0	0
	6	0	0	0	3	0	1	0	1	0	1	0	1
	7	6	3	5	0	2	0	0	1	0	5	2	2
Control 2	1	3	10	2	8	7	5	5	6	5	6	3	2
	2	2	2	4	3	9	9	4	8	7	4	7	1
	3	2	1	5	1	0	0	7	5	7	4	5	1
	4	1	6	5	5	10	6	7	7	6	5	5	2
	5	2	3	3	5	13	12	12	9	9	11	11	8
	6	3	6	3	13	15	15	5	15	15	14	14	1
	7	15	20	13	19	16	11	15	13	15	15	14	15
Experimental 1	1	0	0	0	0	4	6	6	9	5	10	4	5
	2	0	1	2	7	0	0	0	2	1	0	0	2
	3	0	0	7	2	1	0	0	0	0	2	1	2
	4	1	5	12	4	11	11	3	1	0	2	0	0
	5	0	9	0	1	9	9	9	16	13	11	9	9
	6	3	8	0	1	0	9	0	3	0	0	5	0
	7	0	0	0	0	3	3	4	2	2	1	2	1

TABLE 4.8 WATER QUALITY DATA MEASURED DURING EXPERIMENT 1

	Number of Samples	Mean	Standard Deviation	Range
pH	154	8.5	0.1	8.2 - 8.7
Dissolved Oxygen (mg/L)	154	8.4	0.9	6.6 - 10.3
Temperature (°C)	154	19.9	0.3	19.1 - 20.6
Conductivity (umhos)	154	630	14	617 - 669
Turbidity (NTU)	166	4.8	1.2	2.2 - 7.0

TABLE 4.9: TYPICAL FISH RESPIRATORY ACTIVITY BEFORE AND AFTER ARTIFICIAL DAWN AND DUSK PERIODS

Test Fish	Light Dimming		Light Brightening	
	half hour before	half hour after	half hour before	half hour after
	Average Ventilatory Range (cycles/minute)			
Fish 1	54	33	28	46
Fish 2	47	37	37	47
Fish 3	60	42	41	59
	Average Amplitude of Ventilation (volts)			
Fish 1	11	9	8	11
Fish 2	6	5	6	6
Fish 3	16	7	8	13.5

TABLE 4.10: COMPARISON OF NIGHT- AND DAY-TIME FISH RESPIRATORY VARIABILITY

Test Day	Control Fish 1				Control Fish 2			
	Mean Ventilatory Rate (cycles/ min.)	Ventilatory Rate Variability SD as (cycles/ min.)	Mean Amplitude of Ventilation (volts)	Average Range of Amplitude of Ventilation (volts)	Mean Ventilatory Rate (cycles/ min.)	Ventilatory Rate Variability SD as (cycles/ min.)	Mean Amplitude of Ventilation (volts)	Average Range of Amplitude of Ventilation (volts)
Day 5	50	16	11.3	7.6	77	22	7.4	9.7
Night 5	22	2	6.1	3.6	39	2	5.1	1.9
Day 6	42	15	12.7	11.5	56	4	4.5	7.1
Night 6	24	2	4.9	5.4	43	5	3.7	1.7
Day 7	56	11	9	13.8	69	14	4.4	7.6
Night 7	21	2	4.8	6.9	48	9	8.1	3.8

TABLE 4.11: TYPICAL RECOVERY OF RESPIRATORY ACTIVITY FOLLOWING FISH FEEDING

Time of Day	Fish 1			Fish 2			Fish 3		
	Ventilatory Rate (cycles/ min.)	Amplitude (volts)	Cough Rate (cycles/ min.)	Ventilatory Rate (cycles/ min.)	Amplitude (volts)	Cough Rate (cycles/ min.)	Ventilatory Rate (cycles/ min.)	Amplitude (volts)	Cough Rate (cycles/ min.)
1000	43	2.9 - 3.6	0	88	0.5 - 3.6	0	64	0.9 - 2.1	0
1213	66	1.2 - 3.3	0	109	0.6 - 1.7	0	59	0.8 - 1.3	0
1215[1]	86	1.1 - 4.1	0	126	2.3 - 4.5	0	67	0.3 - 3.4	5
1218[2]	71	1.7 - 4.5	0	113	1.6 - 4.5	0	63	0.7 - 2.0	6
1220	67	1.0 - 3.5	0	107	1.4 - 3.5	0	60	0.5 - 2.1	0
1223	62	0.7 - 2.9	0	99	0.5 - 2.2	0	44	0.8 - 2.2	0

[1]start of feeding period
[2]end of feeding period

Respiratory rates for the different fish were variable as shown in Table 4.12 under the column Test Fish. For 75% of the time variation in respiratory rates between fish at the same time of day was greater than for individual fish for different days but for the same time period. For light and dark periods, 52% and 91% respectively of the variances for respiratory rates for individual fish were less than those between fish. Therefore comparison of a fishes own activity would narrow the limits defining abnormal respiratory activity and would increase the sensitivity of a monitoring system. Also, the wide range in normal respiration of an unstressed fish demonstrated the importance of having a large number of fish to provide statistical confidence and to overcome the effects of unresponsive and over-responsive fish.

Based on the plots of fish respiratory activity, there was no evidence to suggest that the single fish exposed 3,250 ug/l chloroform and 50 ug/l bromodichloromethane altered its activity from that prior to exposure to chloroform (Table 4.13) or from that of the two reference fish (Appendix). Since this chloroform concentration is an order of magnitude higher than the highest levels reported in raw or finished drinking water and above drinking water guidelines, it is unlikely that a fish physiograph could be used to monitor safe water quality in drinking water supplies (79, 93). However, this statement relates only to chloroform and is based on only one test.

TABLE 4.12: VARIATION IN RESPIRATORY RATE COMPARED WITHIN AND BETWEEN FISH FOR A 3 DAY PERIOD FOLLOWING ACCLIMATION.

Time of Day Day 5, Day 6, Day 7	Test Fish[1]				Test Day[2]		
	Fish 1	Fish 2	Fish 3		Day 5 Fish 1,2,3	Day 6 Fish 1,2,3	Day 7 Fish 1,2,3
0800	85	703	5		129	845	-
1000	149	271	290		508	236	169
1200	528	967	286		496	94	165
1400	257	124	29		405	120	333
1600	148	21	389		266	32	53
1800	2	72	9		97	19	234
2000	6	216	8		46	16	458
2200	8	100	43		37	159	361
2400	1	45	20		89	144	281
0200	5	9	4		73	138	127
0400	11	5	7		103	114	53
0600	1	25	1		82	159	58

[1]each variance was calculated from determinations of respiratory rate for the same fish at the same time of day but on 3 different days.
[2]each variance was calculated from determination of respiratory rate for 3 fish on the same day and time of day.

TABLE 4.13: EXPERIMENT 1: CHLOROFORM DOSAGE

Date	Hour	Exposure Hour	Fish	Concentration (ug/L)				
				Chloro-form	Bromodichlo-romethane	Trichloro-ethylene	Chlorodibro-momethane	Tetra-chloro-ethlyene
2 Oct. 1980	2100	4	Exposed	2,090	46.0	ND	2.00	1.00
3 Oct. 1980	0000	7	Control	2.80	ND	0.02	ND	0.02
4 Oct. 1980	0700	38	Exposed	3,250	50.0	ND	9.00	1.00
4 Oct. 1980	0700	38	Control	2.41	0.15	0.25	ND	0.03

4.5.2 Experiment 2 Feasibility of Monitoring Variations in Quality of a Drinking Water Supply

Pre-exposure variation

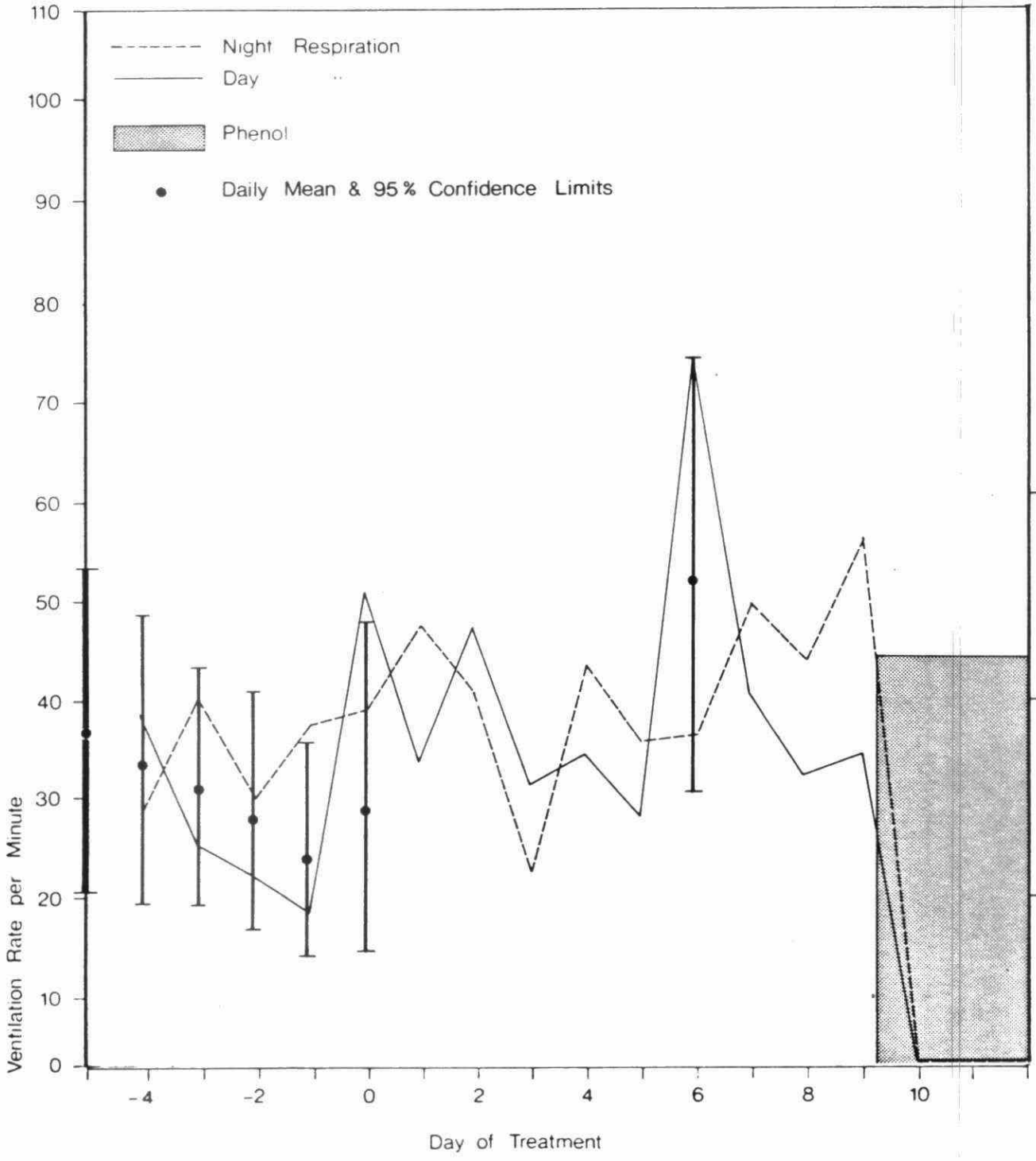
There was variation in the response of the test-fish prior to introduction of the three chemicals selected for this dose-response evaluation as shown in Figures 4.17 and 4.18. These figures illustrates pre exposure variations up to the ninth and eleventh days when the two fish were dosed with phenol. Similar variation was observed with the remaining fish used in dose response experiments (Figures 4.21, 4.25, 4.27, 4.29, 4.30). However, as in Experiment 1, this variation was not attributed to a reaction to ambient levels of compounds occuring in the drinking water source but rather to inherent variation between and within fish. There was no correlation between fluctuations in respiration of the different test-fish either to each other or to changes in the raw water as water quality was relatively stable throughout the experiment. Dissolved oxygen was always greater than 80% saturation, pH and temperature were relatively constant at 8.2 and 16°C and only slight fluctuations in conductivity (587-782 umhos) and turbidity (0.9 - 34 NTU) occured as shown in Table 4.14.

Some of the variation in the respiration was attributed to the diurnal pattern but this variation was less well-defined than in Experiment 1 where night respiration was less variable and lower than in the day. In Experiment 2, three fish had more regular respiratory rates during the day than at night while fish Number 3 was more variable during the day and the remaining fish were equally variable during the night and day. Appendix 3 shows the respiration of each fish every two hours throughout the experiment. This disruption of the rhythm of the fish may have been caused by the lengthening of the night period to 18 hours. This has been substantiated by Gruber who used a 24 hour dimmed light photoperiod to damp diurnal rhythm. Also, the colder water may have reduced the metabolic rate of the fish such that there was no clear active and quiescent periods. Although ventilation rate did not show diurnal changes, the pattern of ventilation was different during the day and night as found in Experiment 1 (Figure 4.19, 4.20).

Ventilatory Response of Phenol Exposed Fish

4.17

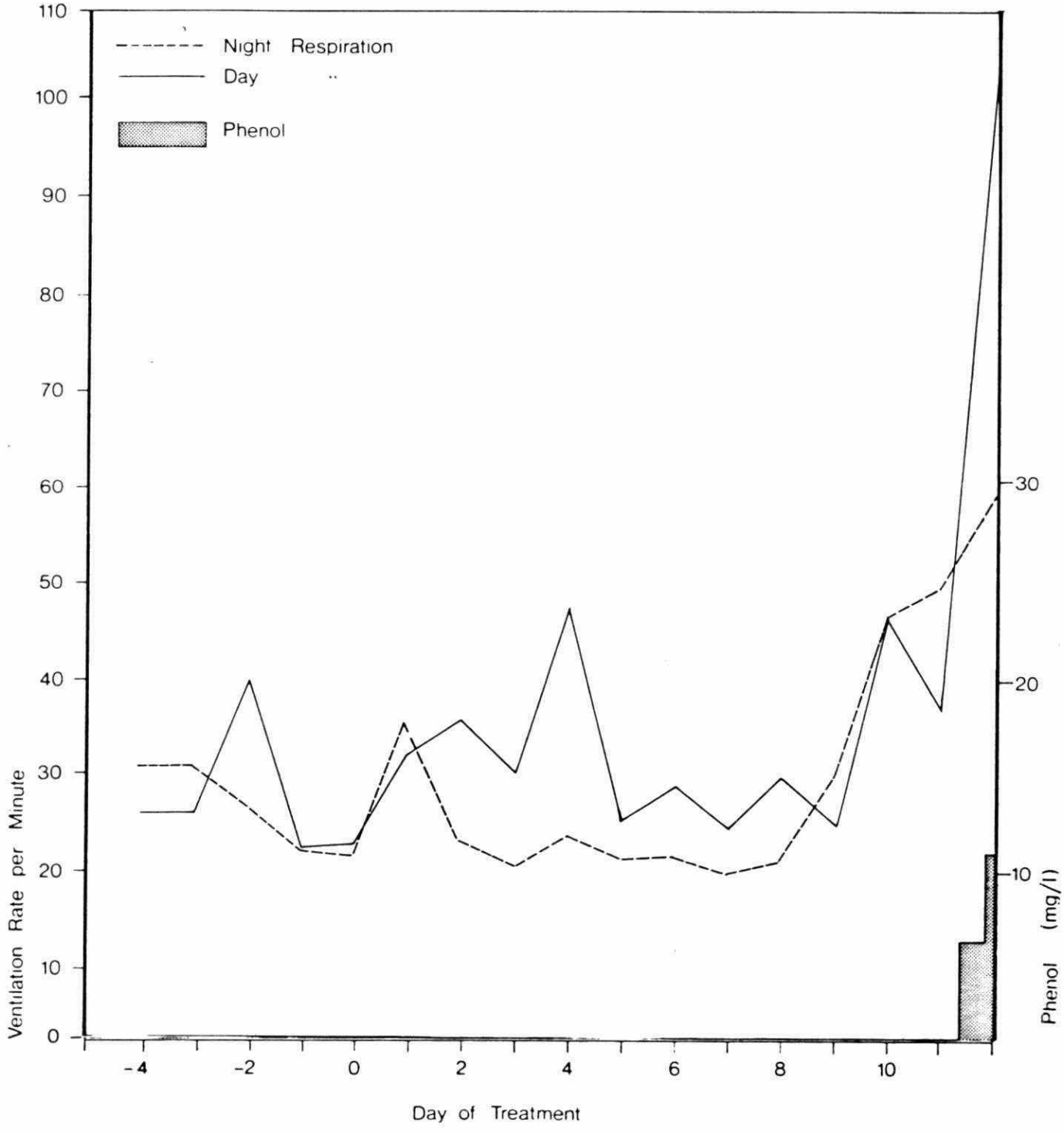
Fish 7



Ventilatory Response of Phenol Exposed Fish

4.18

Fish 8

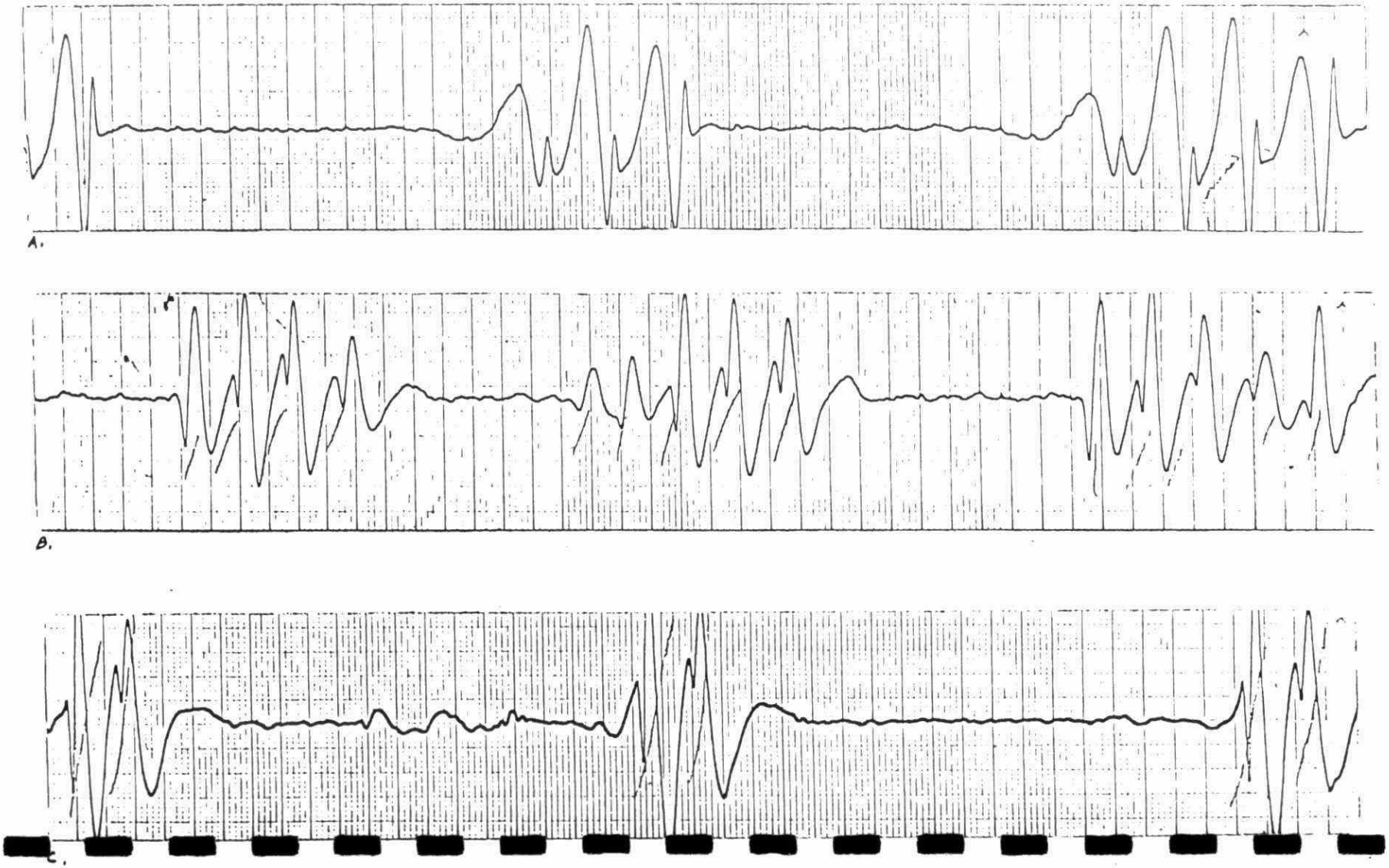


Day Respiration of 3 Largemouth Bass of Similar Size

4.19

Chart speed 10 mm/sec. Sensitivity 200 mV/division.

Note enhanced amplitude of ventilation.



Night Respiration of 3 Largemouth Bass of Similar Size

4.20

Chart speed 10 mm/sec. Sensitivity 200 mV/division.

Note depressed amplitude.

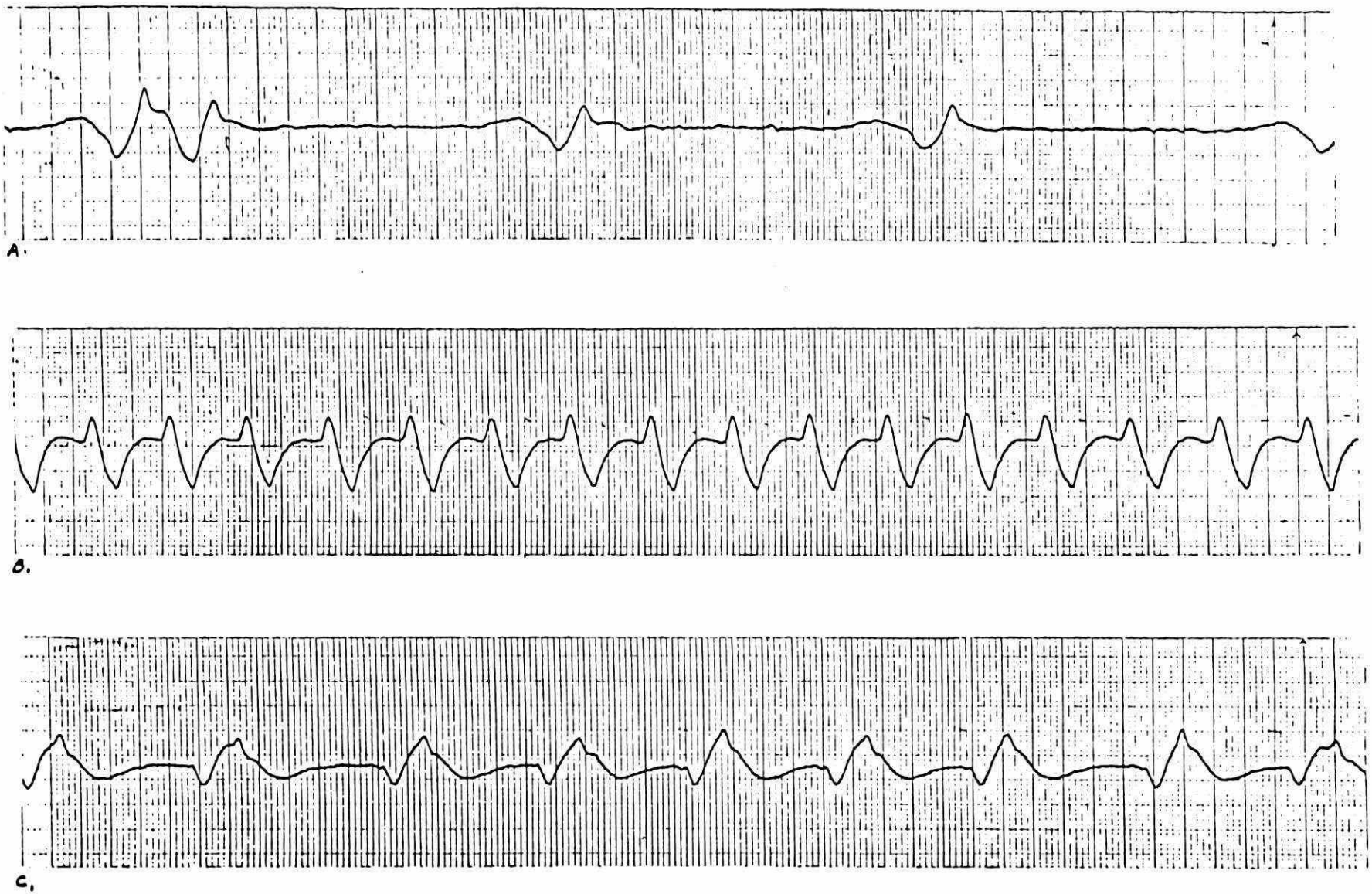


TABLE 4.14 EXPERIMENT 2 WATER QUALITY CHARACTERISTICS

Date (Dec.1980)	Dissolved oxygen (mg/L) N	Mean ± S.D.	pH N	Mean ± S.D.	Temperature (°C) N	Mean ± S.D.	Conductivity (umhos) N	Mean ± S.D.	Turbidity (NTU) N	Mean ± S.D.
2	24	8.7 ± 0.3	24	8.4 ± 0.1	24	16.2 ± 0.1	24	745 ± 3	24	1.7 ± 0.4
3	24	8.9 ± 0.3	24	8.3 ± 0.0	24	15.7 ± 0.1	24	743 ± 7	24	4.8 ± 1.4
4	24	9.1 ± 0.2	24	8.2 ± 0.1	24	15.7 ± 0.1	24	671 ± 35	24	11.4 ± 2.6
5	24	9.1 ± 0.1	24	8.2 ± 0.0	24	15.8 ± 0.1	24	660 ± 9	24	9.4 ± 1.0
6	24	8.7 ± 0.2	24	8.2 ± 0.0	24	15.8 ± 0.1	24	684 ± 5	24	7.3 ± 0.8
7	24	8.4 ± 0.3	24	8.2 ± 0.0	24	16.0 ± 0.2	24	697 ± 3	24	5.5 ± 0.4
8	24	8.5 ± 0.3	12	8.2 ± 0.0	12	16.1 ± 0.1	12	689 ± 3	24	4.3 ± 0.5
9	24	8.6 ± 0.1	13	8.1 ± 0.0	12	16.4 ± 0.0	12	697 ± 9	24	6.1 ± 1.3
10	24	8.6 ± 0.3	24	8.1 ± 0.0	24	16.2 ± 0.1	24	631 ± 31	24	20.0 ± 8.6
11	24	8.5 ± 0.2	24	8.1 ± 0.0	24	16.0 ± 0.1	24	598 ± 11	24	25.4 ± 6.7
12	24	8.0 ± 0.2	24	8.1 ± 0.0	24	16.1 ± 0.2	24	652 ± 13	24	10.6 ± 2.7
13	24	8.2 ± 0.5	24	8.2 ± 0.1	24	16.1 ± 0.2	24	705 ± 14	24	5.9 ± 0.6
14	24	8.8 ± 0.2	24	8.1 ± 0.1	23	16.9 ± 1.7	23	712 ± 10	24	3.4 ± 0.6
15	24	8.7 ± 0.1	24	8.2 ± 0.0	24	15.9 ± 0.5	24	726 ± 21	24	2.6 ± 0.0
16	24	8.9 ± 0.1	24	8.2 ± 0.0	24	15.9 ± 0.5	24	760 ± 6	24	2.6 ± 0.5
17	12	8.6 ± 0.2	9	8.1 ± 0.0	10	15.7 ± 0.6	10	748 ± 1	24	2.0 ± 0.2
18	4	8.6 ± 0.2	24	8.1 ± 0.0	24	15.6 ± 0.5	24	752 ± 4	24	1.9 ± 0.1
19	7	8.3 ± 0.2	24	8.1 ± 0.0	24	15.9 ± 0.5	24	754 ± 1	24	1.8 ± 0.1
20	5	8.3 ± 0.2	24	8.1 ± 0.0	24	16.1 ± 0.4	24	759 ± 4	24	1.6 ± 0.2
21	5	8.2 ± 0.3	24	8.1 ± 0.0	24	15.8 ± 0.6	24	762 ± 4	24	1.4 ± 0.0
23	6	8.0 ± 0.3	24	8.1 ± 0.0	24	15.9 ± 0.5	24	783 ± 2	24	1.2 ± 0.1
24	4	7.5 ± 0.2	17	8.1 ± 0.0	17	15.8 ± 0.6	17	765 ± 6	17	1.2 ± 0.0

Natural respiration variation emphasized the need for control fish and a sufficient number of test-fish so that irregular changes in respiratory activity did not mask real changes. As shown in Figure 4.17 if the pre exposure period (-5 to 0 days) had been used to define normal respiration activity of this reference fish, a false response would have occured at day 6. Other researchers use control fish to account for changes in respiration not associated with changes in quality of the water. IEC also ran similar controls in this experiment. Given the natural variability among fish, van der Schalie (72) recommended using at least 4 fish to minimize false alarms and increase the chance of detecting toxic shifts in water quality. Furthermore, the collection of more than 5 minutes of recordings every 2 hours would provide a better respiration data base. Longer monitoring periods are labour intensive when analysing the data manually but they would be possible using a computerized system. Gruber and Cairns recommend monitoring ten minutes or more for each assessment period to limit the effect of normal variability in ventilatory rate.

Dose-response effect

Fish exposed to sublethal levels of stepwise concentration increments of phenol, ammonia and zinc significantly altered their respiratory activity. Ventilatory rate, cough frequency and amplitude of respiration were all affected by exposure to these introduced chemicals as noted in Table 4.15. Respiration changes occured at fractions of levels acutely lethal to fish. High concentrations produced shorter response times such that respiration changes to acutely lethal concentrations occured in less than two hours. Prolonged exposure to the highest concentrations (Table 4.16) resulted in the death of 4 of the 8 test-fish at the end of the 22 day experiment.

Exposure to 1.5 mg/l phenol provoked an increased cough frequency (Figure 4.21, 4.22) No coughs had occured prior to this exposure. Elevation of the concentration from 1.5 to 4.6 and then 7.9 mg/L resulted in a substantial rise in ventilation rate (Figure 4.21). A further increase in ventilation rate occurred at 11.4 mg/L phenol as shown in Figure 4.23. Figure 4.24 illustrates exposure to 22 mg/L phenol for a 1-5 hour period caused a significant decline in amplitude of ventilation followed by death.

TABLE 4.15: DETECTION LIMITS OF THE EXPERIMENTAL SYSTEM

	Phenol	Ammonia	Zinc
Cough Frequency	increased at ≥0.3≤1.5 mg/l within 72 h	no response at <70 mg/l after 22 h	increased at ≥0.5≤1.3 mg/l within 48 h
Ventilatory rate	increased at ≥1.5≤4.6 mg/l within 8 h	no significant change after 22 h	decreased at >0.8<4.6 mg/l within 2 h
Amplitude	increased at <11.4 mg/l and then decreased at <22 mg/l with 4 h	increased at ≥10≤20 mg/l and then decreased at ≥10≤20 mg/l within 7 h	decreased at ≥1.1<4.8 mg/l within 2 h
Lethal concentrations	Survived exposure to 11.4 mg/l for 7 hours. Died after 1-5 hours exposure to 22 mg/l.	One fish died after 7 hours exposure to 73 mg/l total NH_3 or 2.1 mg/l unionized NH_3 (16°C, pH 8.1).	Survived exposure to 15.5 mg/l for 5 hours.

TABLE 4.16: CHEMICAL ANALYSIS OF EXPOSURE WATER (mg/l)

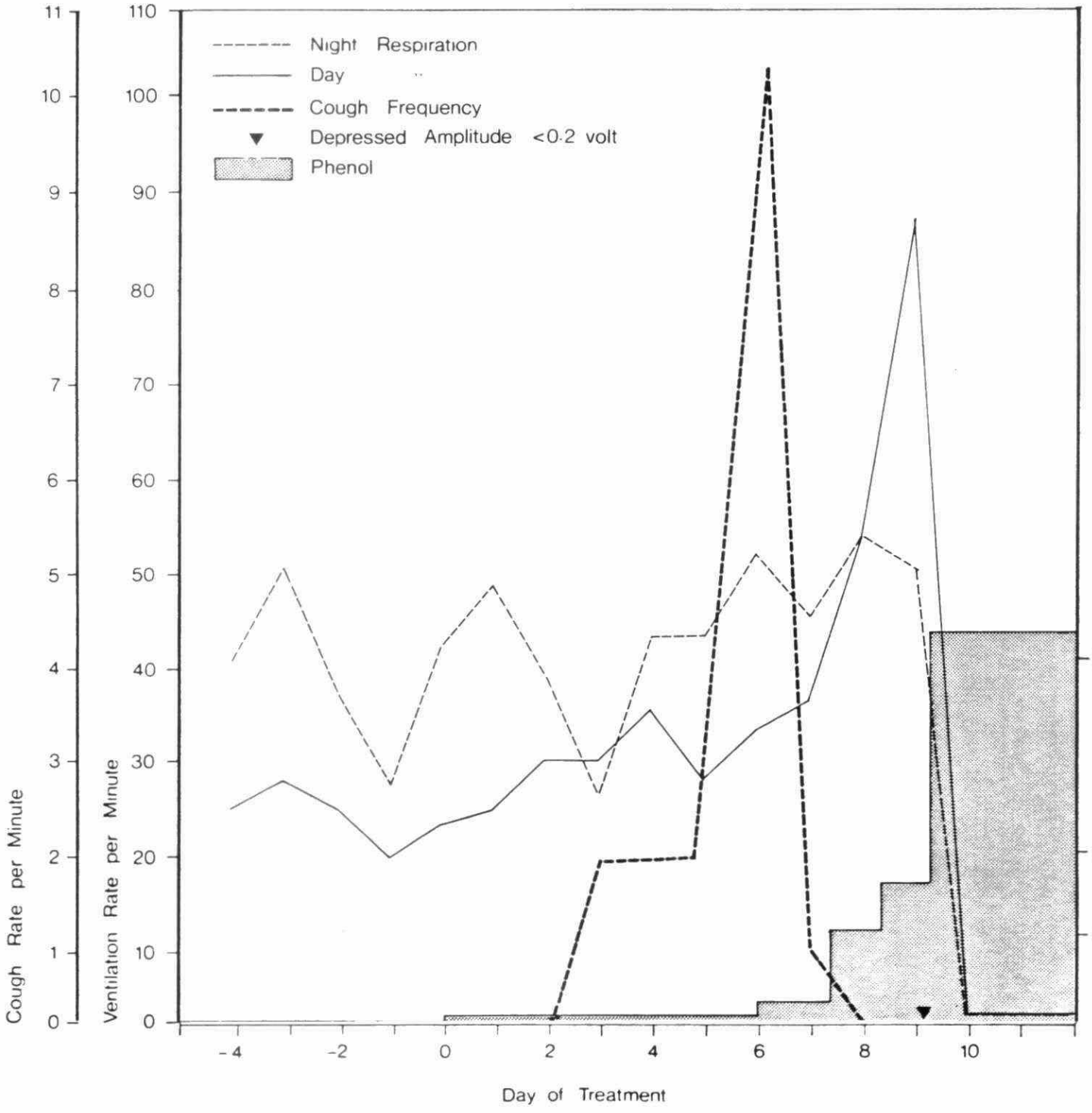
Date Time	Test-Fish					
	1	2	3	4	5	7	8
(Dec. 1980)	Phenol	Total Ammonia	Total Ammonia	Zinc	Zinc	Phenol	Phenol
2-12 1800	<.0005	<.05	<.05	<.01	<.01	<.0005	<.0005
13 0300	<.0005	1.2	0.8	<.01	<.01	<.0005	<.0005
13 1700	0.290	1.2	1.0	0.81	<.01	<.0005	<.0005
14 1900	-	0.7	1.2	-	-	<.0005	<.0005
14 2000	0.142	-	-	0.47	0.45	<.0005	<.0005
15 1500	0.146	0.3	0.4	0.41	0.39	<.0005	<.0005
16 0800	-	-	0.2	-	-	<.0005	<.0005
16 1530	0.399	1.6	2.1	1.45	1.45	<.0005	<.0005
17 1800	0.430	1.6	2.4	1.15	0.84	<.0005	<.0005
18 0900	1.470	2.1	2.6	4.85	4.55	<.0005	<.0005
19 1000	1.530	2.7	4.6	4.65	4.65	<.0005	<.0005
20 1100	4.570	2.7	4.6	4.65	4.65	<.0005	<.0005
21 1000	7.940	9.7	12.9	0.01	0.02	<.0005	<.0005
22 1130	21.90	-	-	-	-	22.000	<.0005
24 0900	-	21.2	27.9	9.00	12.90	-	6.120
24 1130	-	61.7	72.8	15.40	15.60	-	-
24 1600	-	-	-	-	-	-	11.400

- denotes no determination

Ventilatory Response of Phenol Exposed Fish

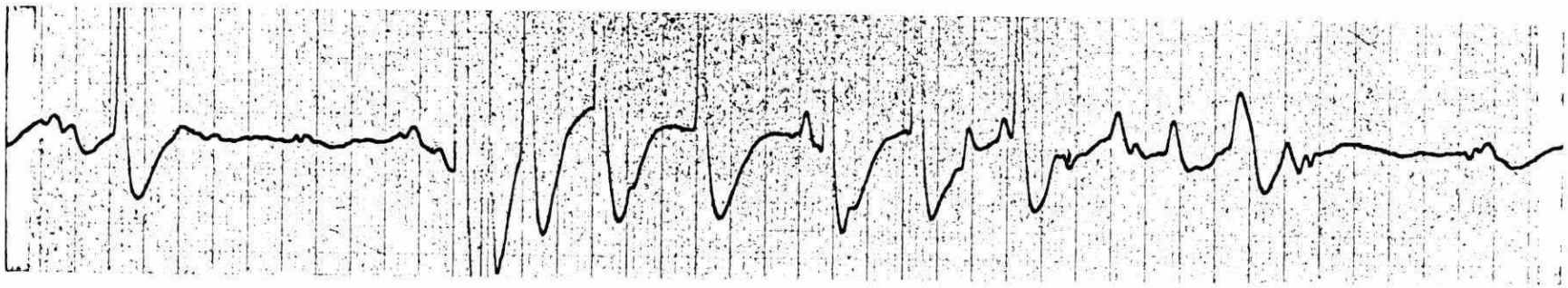
4.21

Fish 1

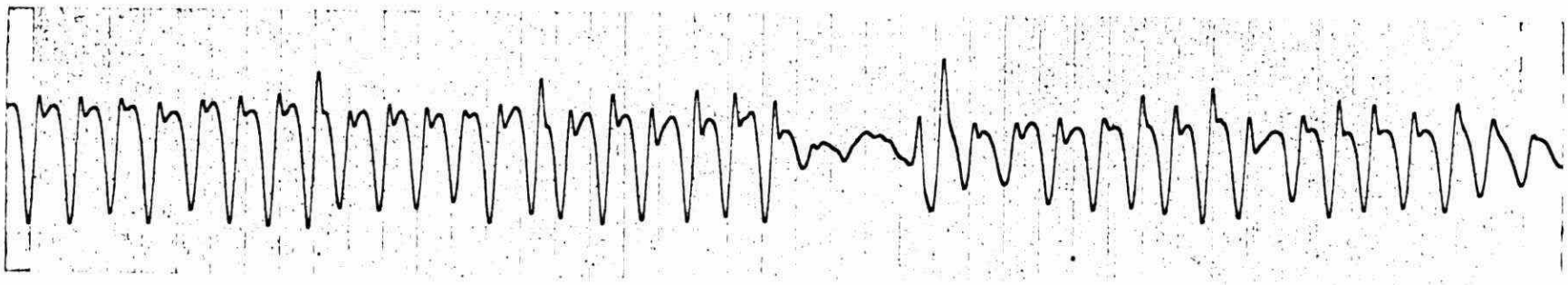


Respiratory Response of Largemouth Bass to Phenol

4.22



A. Phenol 1.5 mg/L. Increased cough frequency.

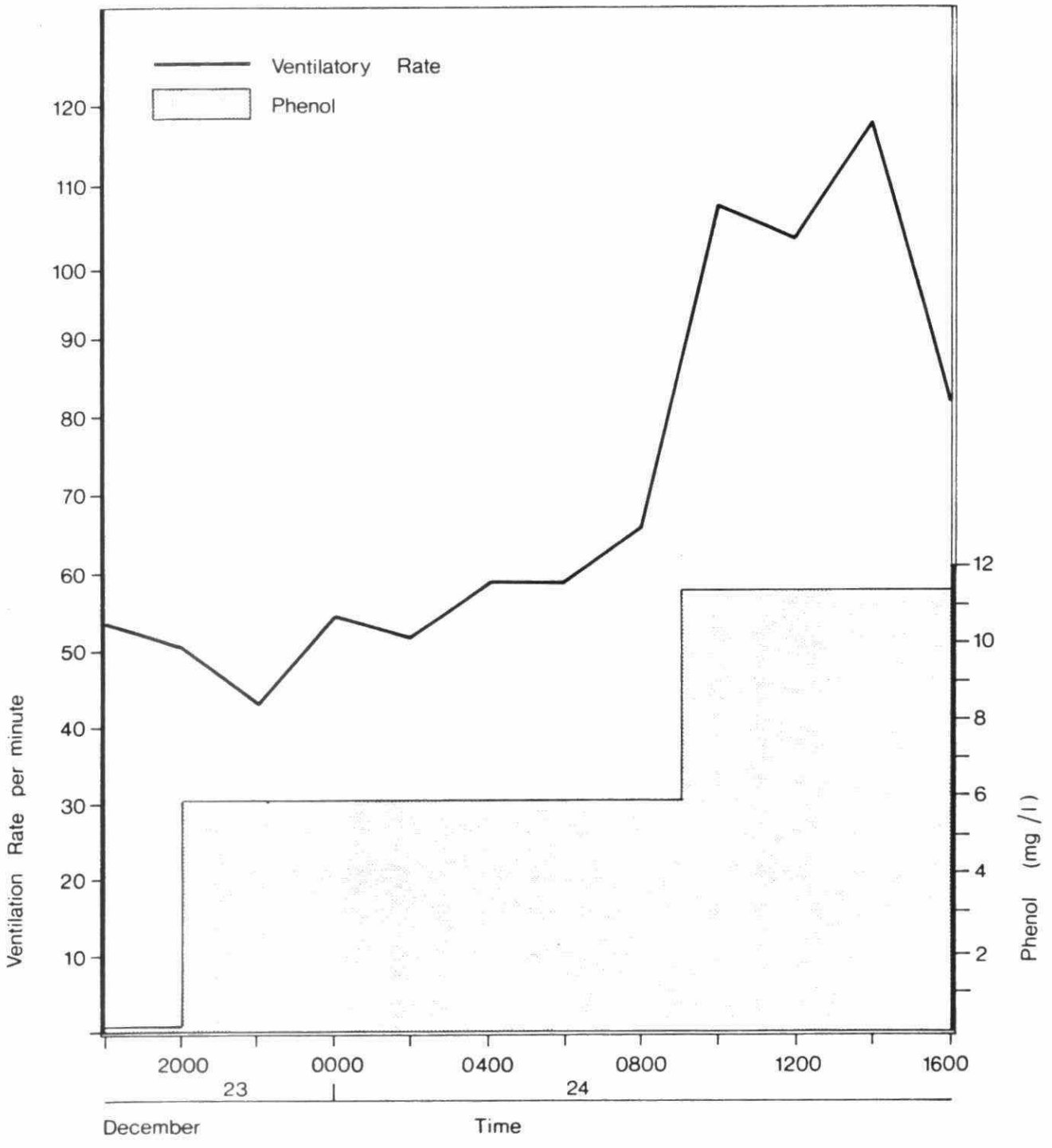


B. Phenol 7.9 mg/L. Increased ventilatory rate.

Short Term Ventilatory Response of Phenol Exposed Fish

4.23

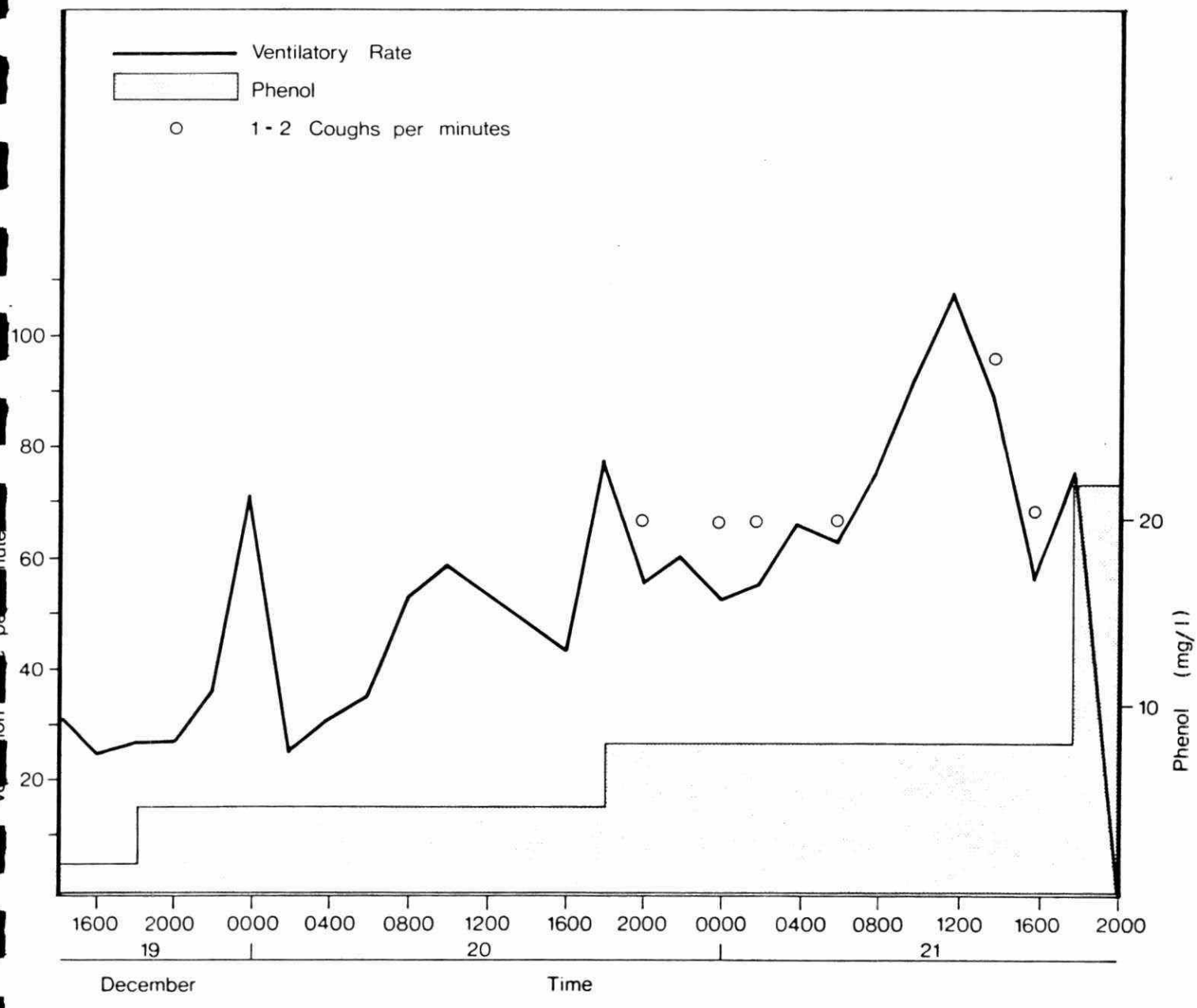
Fish 8



Short Term Ventilatory Response of Phenol Exposed Fish

4.24

Fish 1



Ventilatory response to zinc was similar to phenol. Initial cough response by one of the two exposed fish occured at 1.3 mg/l (Figure 4.25, 4.26, 4.27). This variability of fish cough response to zinc has been previously reported by Sparks (30). Increasing zinc concentration to 4.6 mg/l caused a sharp decline in respiratory amplitude to levels which could not be recorded as illustrated in Figure 4.28. This elevation in concentration also caused a further rise in cough frequency. Removal of the toxicant resulted in an almost immediate return of the ventilatory signal. Subsequent re-exposure with 2.3 mg/l caused a repeat decline in amplitude of ventilation. The final concentration increment to 15.5 mg/l for 5 hours was survived by both fish.

The pattern of response to ammonia was different than to zinc and phenol. Initially, there was no cough response (Figure 4.29, 4.30). Also, there was no increase in ventilation rate and there was a trend towards a slight decline in respiration. The most significant response was a change in respiratory pattern from the usual day and night pattern (Figures 4.19, 4.20) to one of multiple ventilations with extended apneic period of over 20 seconds between breaths (Figure 4.31). Also, amplitude of ventilation was elevated at exposures greater than 10 mg/l and then amplitude declined at 73 mg/l. The final concentration of 73 mg/l was lethal to one of the two exposed fish in less than 7 hours.

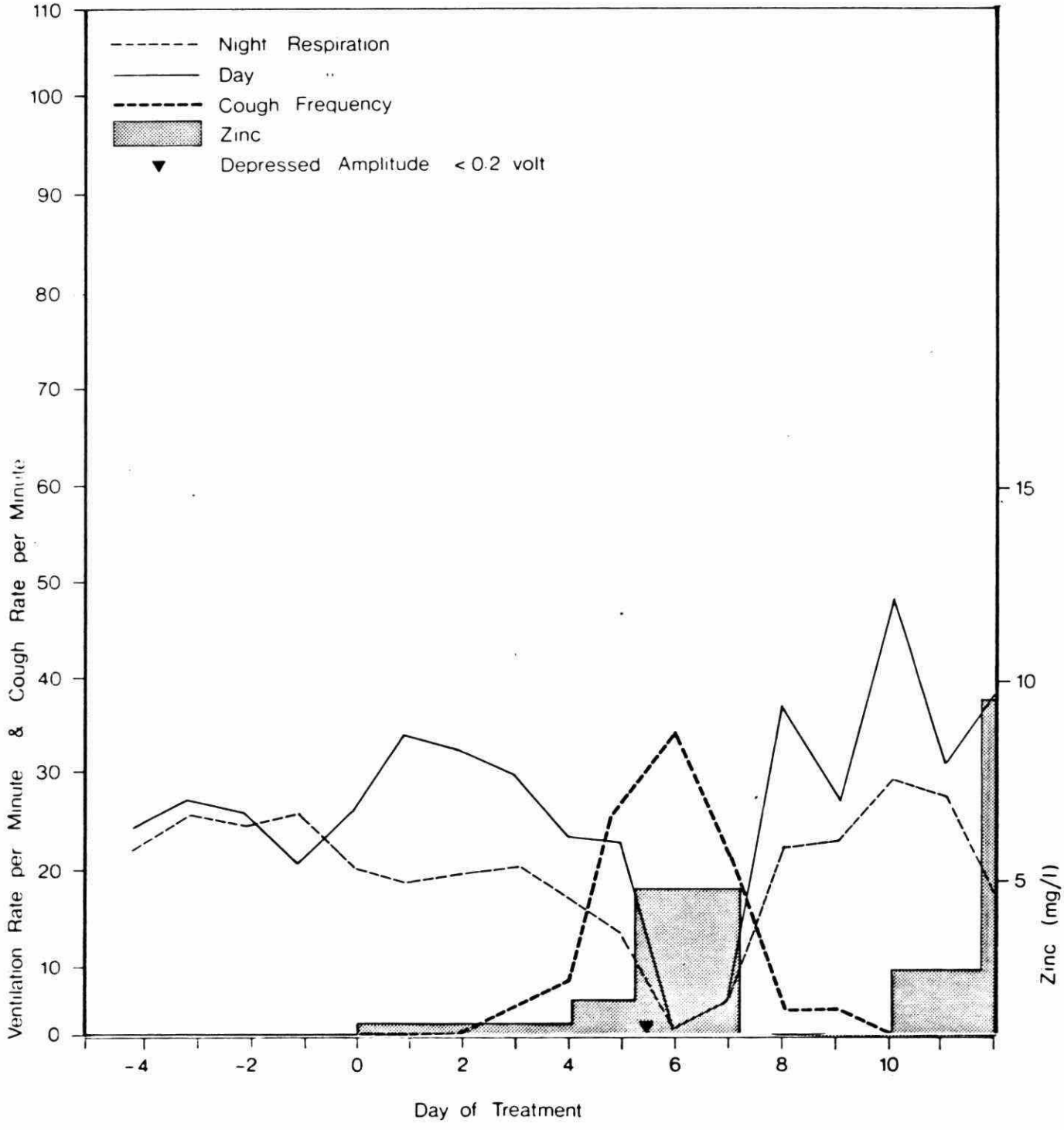
The detection limits and response times for the three compounds were similar to those reported by other researchers (Table 4.17). As reported from other fish physiograph studies, response to zinc was the most rapid and sensitive followed by phenol and then ammonia. The concentrations which elicited alterations in respiration were fractions of acutely lethal levels (Table 4.18).

The most sensitive respiratory parameter was cough response. Ventilatory rate changes were noted at concentrations of 10 and 25% of the 96 hour LC50 for zinc and phenol, respectively. There was no cough response to ammonia, even at lethal exposure levels. Similar findings had also been reported by Smart (94).

Ventilatory Response of Zinc Exposed Fish

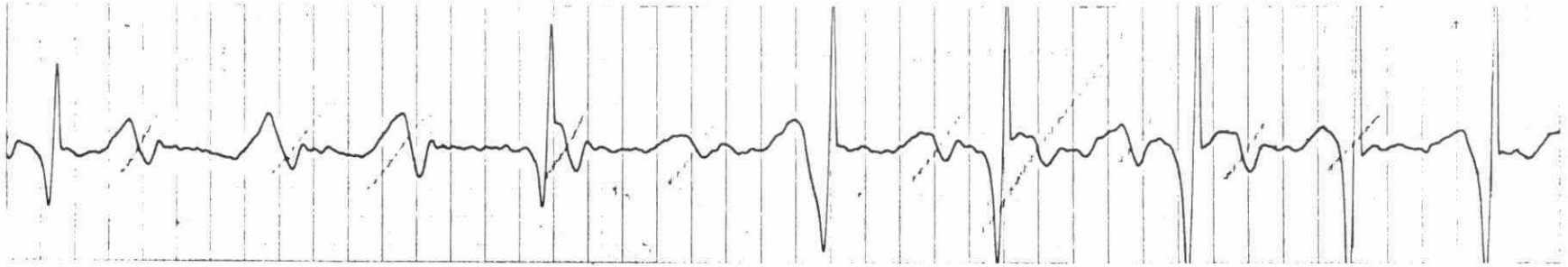
4.25

Fish 4

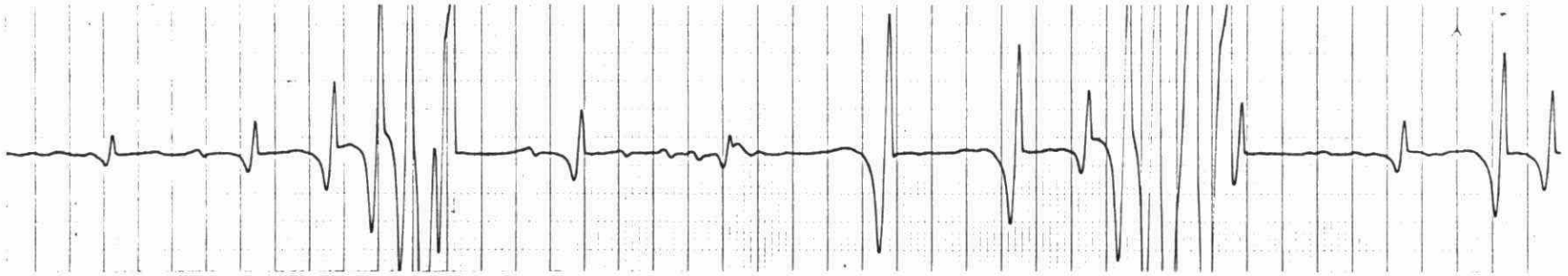


Respiratory Response of Largemouth Bass to Zinc

4.26



A. Zinc 1.3 mg/L. Increased cough frequency.

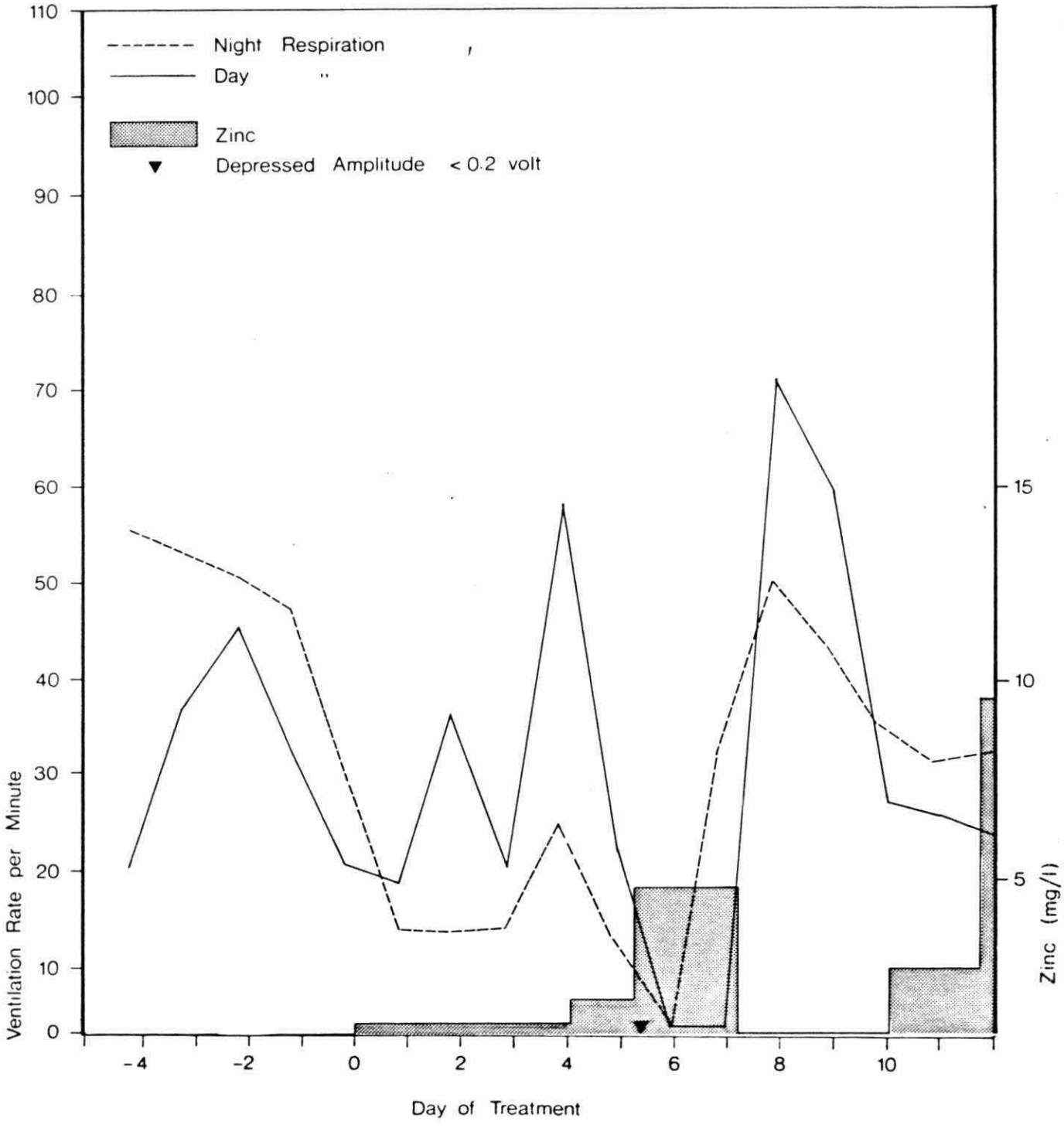


B. Zinc 4.7 mg/L. Increased cough frequency, decreased amplitude, no discernible ventilations.

Ventilatory Response of Zinc Exposed Fish

4.27

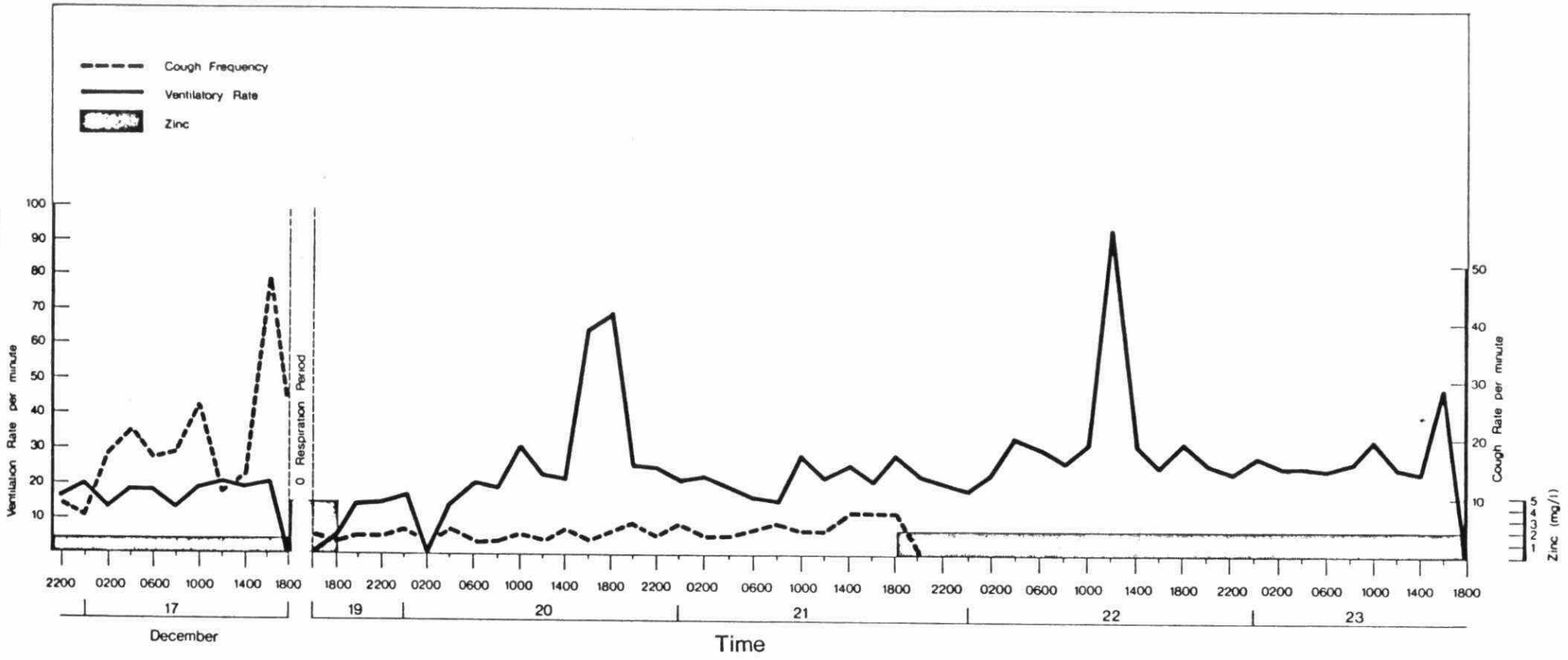
Fish 5



Short Term Ventilatory Response of a Zinc Exposed Fish

4.28

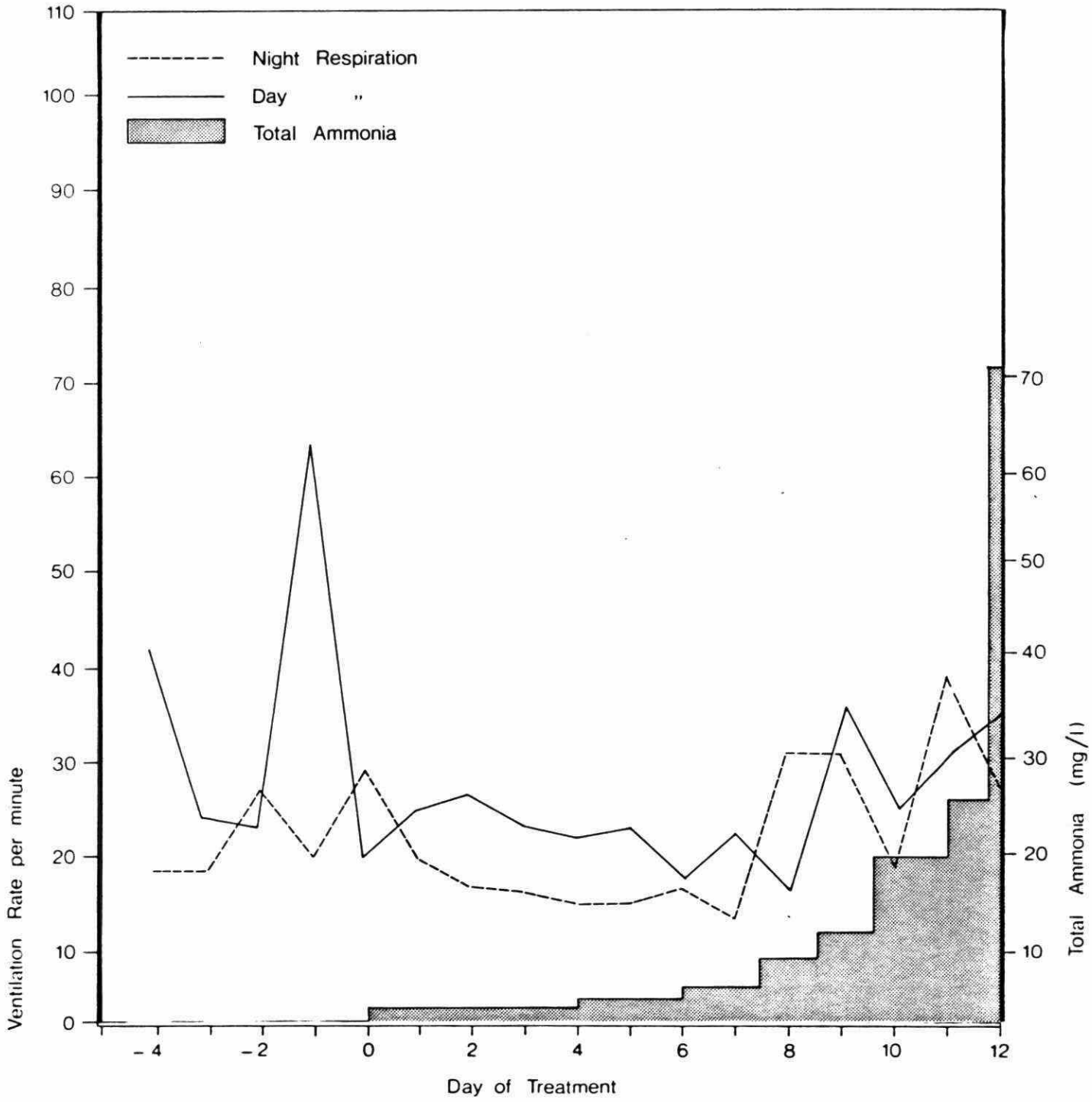
Fish 4



Ventilatory Response of Ammonia Exposed Fish

4.29

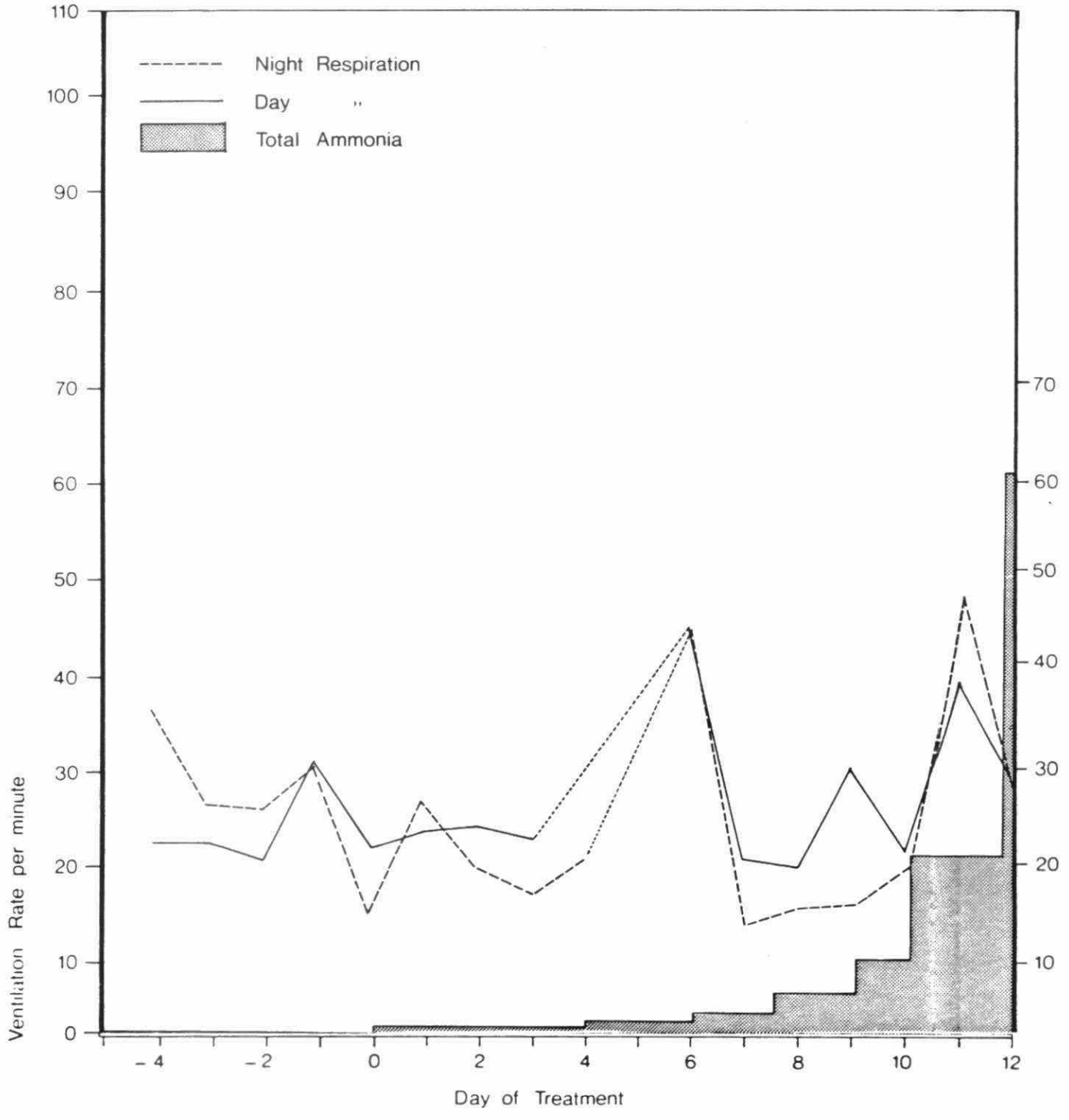
Fish 3



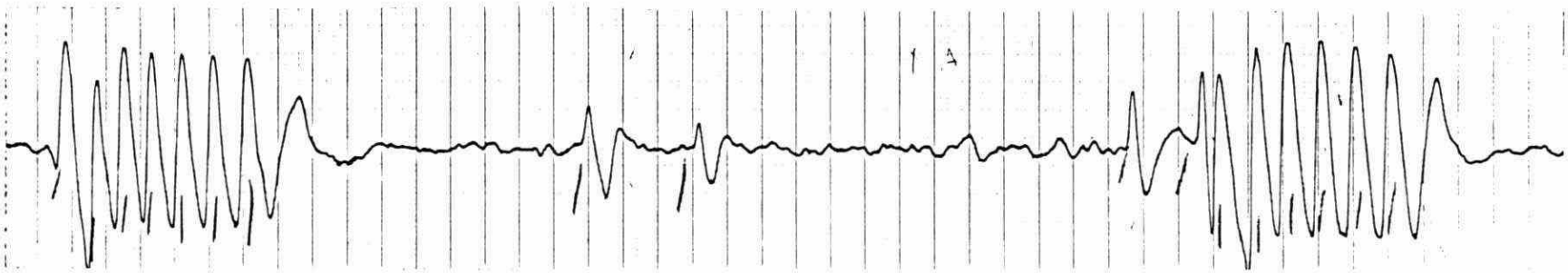
Ventilatory Response of Ammonia Exposed Fish

4.30

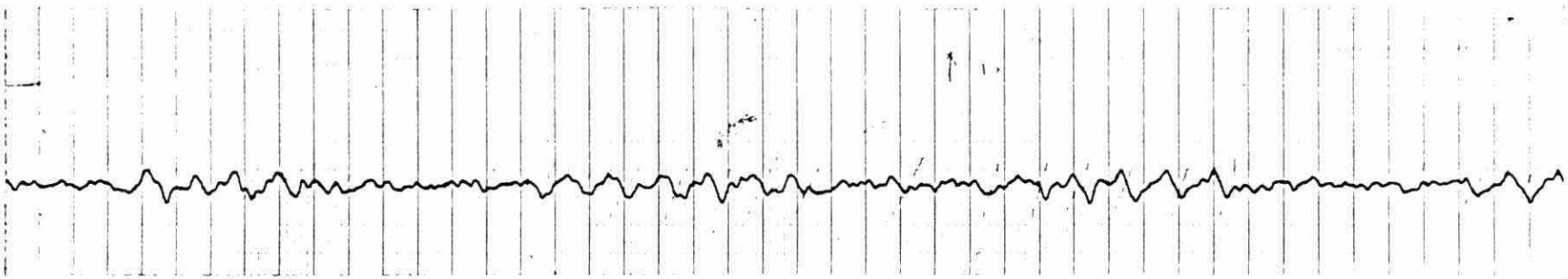
Fish 2



Respiratory Response of Largemouth Bass to Ammonia



A. Total ammonia 20 mg/L $\bar{N}$/L. Multiple ventilations, increased amplitude and apneic period.



B. Total ammonia 73 mg N/L. Multiple ventilations, decreased amplitude and increased apneic period.

TABLE 4.17: DETECTION LIMITS OF FISH PHYSIOGRAPH SYSTEMS TO PHENOL, AMMONIA AND ZINC

Reference	Test Species	Phenol	Ammonia	Zinc
(71)	Largemouth bass	10 mg/l, <1 hour to >respiration	10 mg/l, 7 hours to increase respiration	
		5 mg/l, 6 hours to >respiration	5 mg/l, 12 hours to increase respiration (30% 48h LC50)	
		1 mg/l, 18 hours to >respiration (5% 48h LC50)	1 mg/l, 33 hours	
(49)	Rainbow Trout	4 mg/l, <24 hours to >respiration		
(59)	Bluegill sunfish			0.53 mg/l <1/2 hour to >respiration and <amplitude
(95)	Bluegill sunfish			2.55 mg/l <52 hours to >respiration

Acutely lethal concentrations of phenol, ammonia and zinc

Phenol	Ammonia	Zinc
Bluegill sunfish 24 h LC50 28-60 mg/l 48 h LC50 8-22 mg/l 96 h LC50 6-20 mg/l <temperature>toxicity (96, 97)	for non-salmonid species 24 h LC50 0.6 mg/l UIA 96 h LC50 0.35-1.24 mg/l UIA >pH,>temperature>toxicity Ball (1967) (98, 99)	Rainbow trout 96 h LC50 7.2 mg/l Bluegill sunfish 24 h LC50 10 mg/l 96 h LC50 10-12 mg/l >hardness<toxicity (99, 100)

TABLE 4.18: SUMMARY OF RESPONSES

Respiratory Parameter	Compound		
	Phenol	Zinc	Ammonia
Cough	25% 96 h LC50 <72 h	10% 96 h LC50 <48 h	no response
Ventilation	≤ 100% 96 h LC50 2 - 18 h	25-50% 96 h LC50 <2 h	possible depression
Amplitude	≤ 100% 96 h LC50 <4 h	50% 96 h LC50 <2 h	200% 96 h LC50 <5 h

Ventilation rate was another sensitive parameter for monitoring phenol and zinc. Responses were rated at between 25 and 50% of the 96 hour LC50 within two hours for zinc and <100% of the 96 hour LC50 for phenol in less than 18 hours. Ammonia had a narcotic effect on the fish and ventilation rate declined slightly, but not significantly (Appendix 3). This was contrary to the results of a study by Morgan and Kuhn who observed increased ventilations at 5 and 10 mg/l total ammonia (71). In their study, ammonia was applied as a sudden spike and not gradually elevated in concentration as in this experiment. This difference in mode of toxicant application may explain the variation in reaction by the fish.

Amplitude of ventilation declined at higher concentrations. At 50% and $\leq$100% of the 96 L LC50 for zinc and phenol, respectively this response occurred in less than 4 hours. For ammonia, this reaction occurred just prior to death of one of the fish at 73 mg/l total ammonia, a concentration of about twice the 96 L LC50 for test pH and temperature.

4.5.3 Experiment 3 Effect of Zinc on Rainbow Trout Respiration

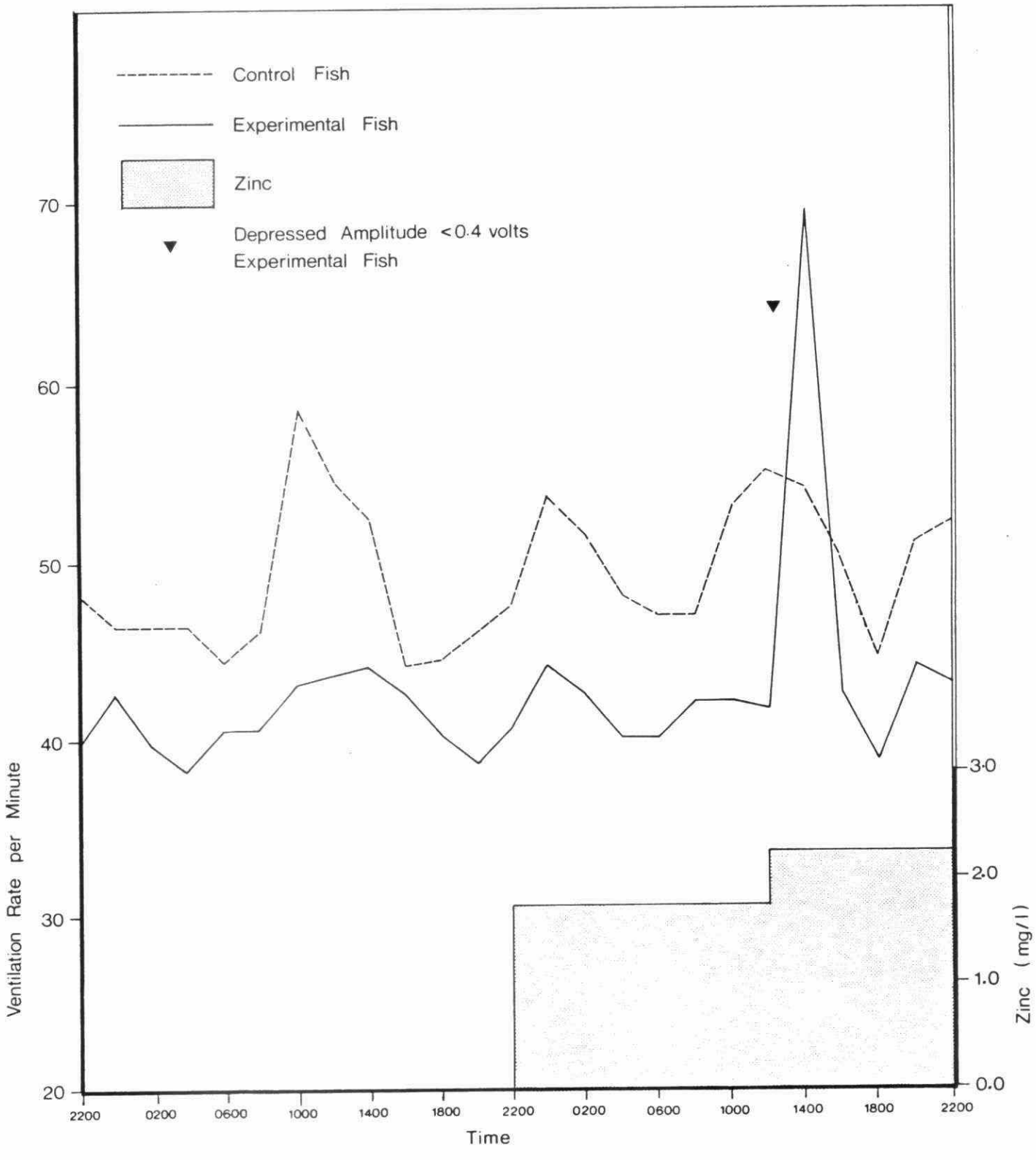
Water quality was uniform throughout Experiment 3. Dissolved oxygen was maintained above 80% saturation, pH was 8.2, temperature was 5 ± 1.0°C., conductivity ranged from 690-734 umhos and turbidity was 1.1-2.6 NTU.

The rainbow trout did not respond to 1.7 mg/l zinc during the initial 14 hours of exposure. However, elevation of the concentration to 2.3 mg/l zinc resulted in a significant reduction in amplitude of ventilation from 4.0 to <0.4 volts and a 35% rise in ventilation rate in less than 2 hours (Figure 4.32 and 4.33). This represented a rapid response to a concentration less than 32% of the 96h LC50 as noted in Table 4.17. This response was only temporary, and within 4 hours ventilatory rate had returned to initial levels. This suggested that fish may react to concentration change rather than actual concentration at low levels. This points to the need for more frequent evaluations of the data to detect subtle changes in water quality. The sensitivity of this zinc response was similar to that observed with largemouth bass and confirmed the suitablity of rainbow trout for monitoring cold water supplies.

Swimming activity may sometimes mask the ventilatory signal recording of rainbow trout as shown by Figure 4.34. This did not occur when using largemouth bass which use pectoral fins and not full body flexure to swim in the cell. Also, rainbow trout have a usual cough rate of 1-2 purges/minute (Figure 4.34) unlike largemouth bass which rarely cough unless stressed. However, the respiratory pattern of rainbow trout was more uniform than that of largemouth bass making trout more amenable to the Fast Fourier Transformation analysis. Signal records of the respiratory pattern of a 0.4 gram rainbow trout illustrated the potential for using sensitive, early life stages in water quality monitoring (Figure 4.34).

Respiratory Response of Rainbow Trout

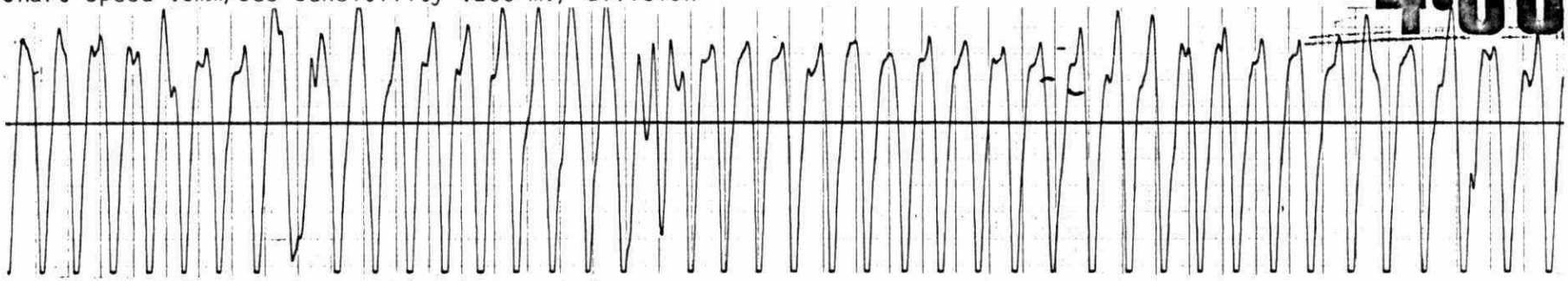
4.32



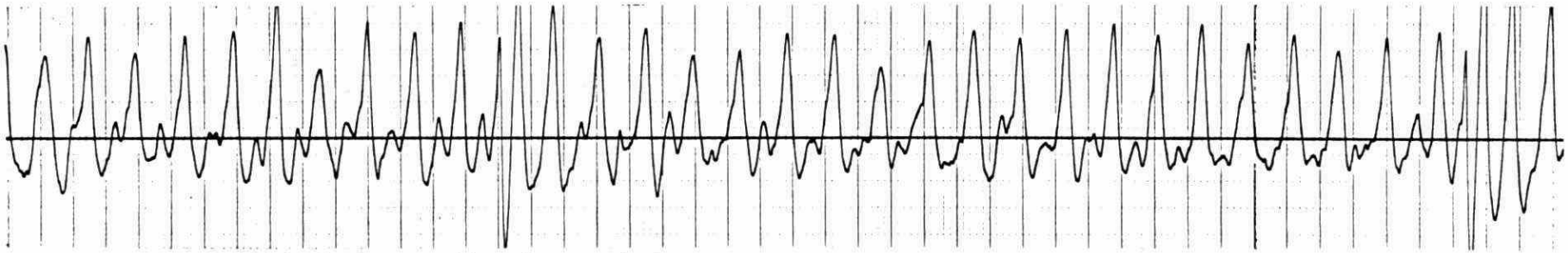
Respiratory Response of Rainbow Trout to Zinc Experiment

4.33

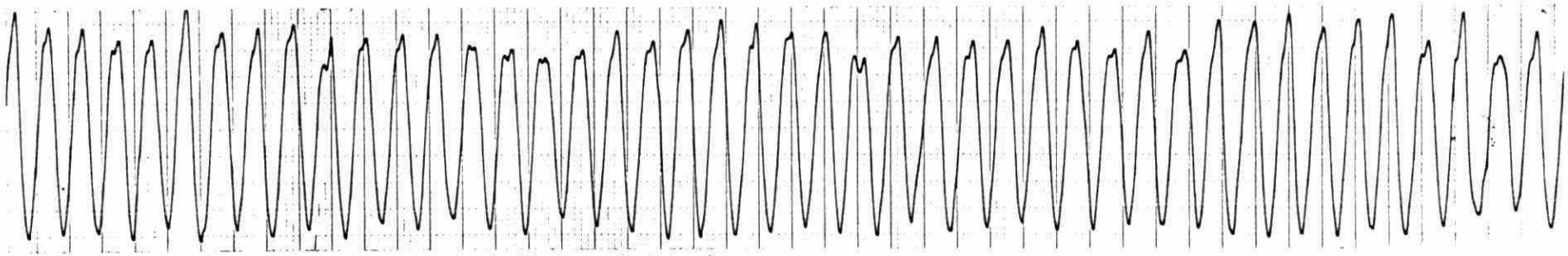
Chart speed .5mm/sec Sensitivity .200 mV/ division



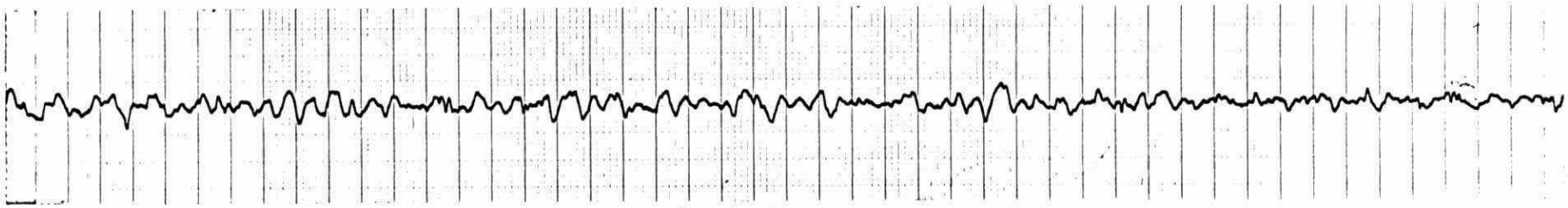
A. Control fish during acclimation period.



B. Experimental fish during acclimation period.



C. Control fish during dose period (0.0 mg/L Zinc).

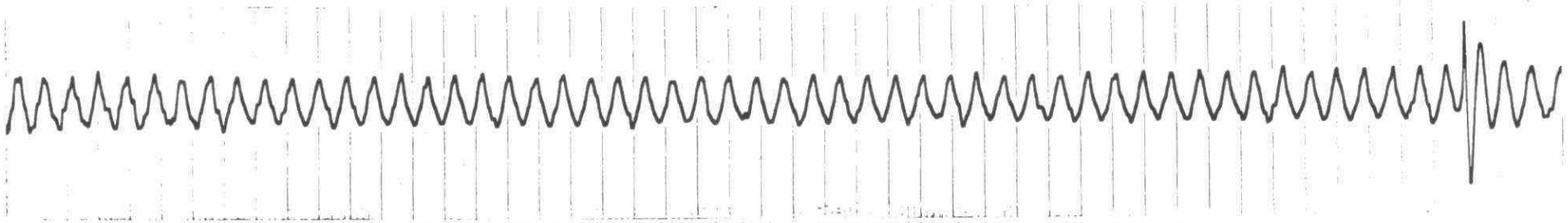


D. Experimental fish during dose period (2.3 mg/L Zinc).

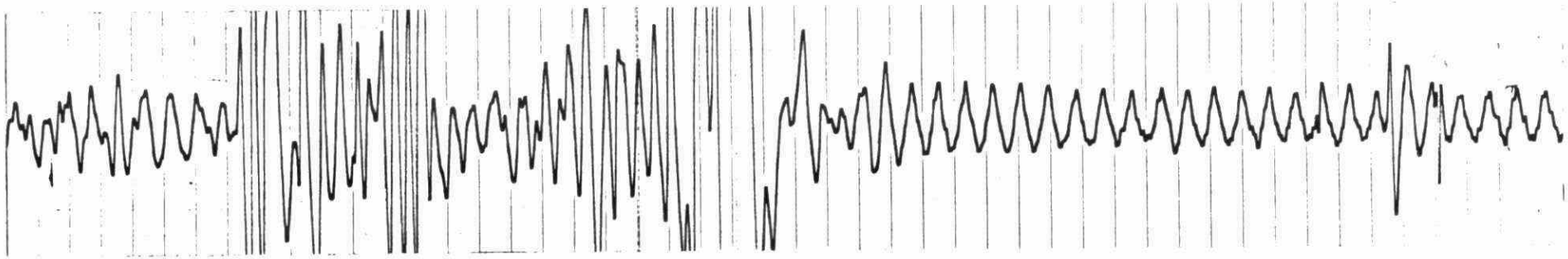
Respiratory Activity of Acclimated Rainbow Trout

4.34

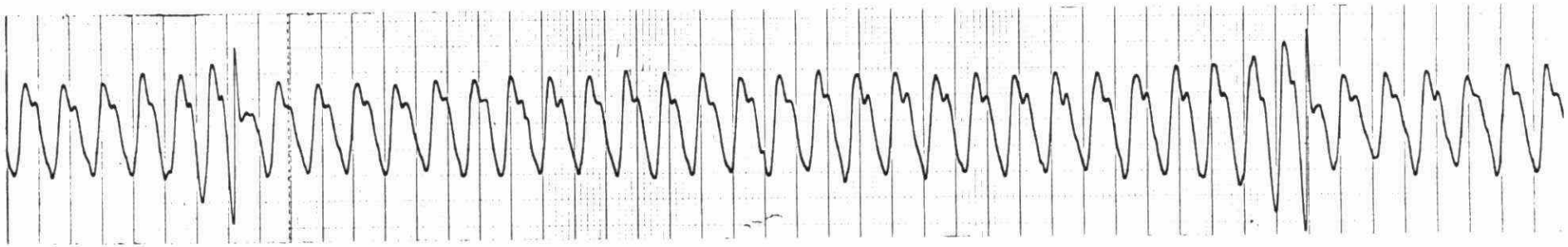
Chart speed 5 mm/sec



A. Rainbow trout 0.4 grams. Note gill purge. Sensitivity 100 mm/division. No Swimming



B. Same fish as above. Illustrates masking of respiratory recordings by swimming activity.



C. Rainbow trout 8 grams. Sensitivity 200 mV/division.

4.6 RECOMMENDED FISH PHYSIOGRAPH APPROACH FOR FUTURE RESEARCH

This section presents a detailed description of recommended fish physiograph system for continuous monitoring and evaluation of water quality. The recommended system is based on IEC field experience and review of systems used by others.

4.6.1 A Strip Chart Monitoring System

This system is designed for computer interfacing.

Test species

Use largemouth bass for summer and fall monitoring and the cold water species, rainbow trout, in winter and spring. Largemouth bass are inactive in cold water and therefore less effective as monitors. This approach would also alleviate problems of water temperature adjustment. Both species are readily available from commercial hatcheries.

Size of fish

Use small fish of 3 to 8 grams and 5 to 10 cm length. Fish of this size emit a signal of low noise to strength ratio and are at a relatively sensitive life stage.

Number of fish

Use two banks of fish, if possible, in practical monitoring, one bank of acclimating fish exposed to control water and the other monitoring bank of fish either exposed to experimental or control water. Each bank should consist of 8-16 fish. The preferred situation would be the following:

MONITORING BANK												ACCLIMATING BANK											
E	E	E	E	E	E	E	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
1	2	3	4	5	6	7	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11	12
EXPERIMENTAL WATER							CONTROL WATER					CONTROL WATER											

C = control or acclimating fish.
E = experimental fish.

The two bank system allows acclimation of fish without any loss in monitoring time. Eight to sixteen fish per bank provide confidence in the statistical determination, allows for unresponsive fish and enables comparison of experimental fish to control fish to examine the acceptability of the test and alarm situations. Furthermore the large number of sensors increases sensitivity by decreasing the width of the confidence intervals. In the event that control water is unavailable, a single bank of six or more fish should be used to monitor the quality of the experimental water.

Electrodes

Stainless steel wire or plate electrodes can be used and oriented either above and below or in front or in back of the fish.

Exposure cell

Two recommended designs are described by Capute and Gruber (1, 2).

Signal carrier cable

Any two solid conductor 22 gage shielded cable is suitable (eg. Belden 8450).

Amplifier/filter and power supply

The noise-immune high gain amplifier/filter described by Gruber is recommended with a 12V DC power supply (3).

Recording device

Use a multi-channel linear strip chart with millivolt and volt sensitivity selector.

Vibration/sound proof enclosure

The unit should be constructed of plywood with 10.1 cm insulated walls and supported on sand-filled boxes.

4.6.2 COMPUTER AUTOMATED SYSTEM

Two computer approaches are presented and outlined in Table 4.19. Both systems monitor water quality on the bases of changes in fish respiratory rate. The computer would sample the respiratory waves of each fish at 25 Hz and estimate respiratory rate using a running average technique by which consecutive positive and negative pulses represent 1 respiratory cycle. The two systems are the VPI & SU system - a proven, well-documented but relatively inexpensive minicomputer system and the North Star system - an unproven but capable, relatively inexpensive, portable microcomputer system.

TABLE 4.19: TWO COMPUTER SYSTEMS

	Minicomputer System	Microcomputer System
Computer	DEC PDP 11/V03-L Model SRBX-SSB 32K	North Star Horizon 780 Model 64K -2D
Commercial software	DEC RT/11-36	CP/M Operating System
	Fortran (no listings)	Microsoft Fortran
Custom software	Fortran programs plus source listing documentation, user guide	To be developed
A/D converter	32 channel and real time clock	2,16 channel and 1 real time clock
D/A converter	2 channel	4 channel
Terminal/printer	Decwriter LA36	Decwriter
Annual maintenance contract		
Supplier	Hamilton Avnet Electronics, Malton 3688 Nashua Drive (416) 677-7432	Computerland, Burlington 678 Guelph Line (416) 632 - 5722
Contact consultant	Dr. D. Gruber VPI & SU	Mr. D. Genner ICS, University of Guelph
Reference	Dr. J. Cairns, Jr. VPI & SU	Dr. E.D. Stevens University of Guelph

4.6.3 BIOLOGICAL PROCEDURES

These recommended procedures apply to both the strip chart system and the fully computer automated system.

Acclimation

Following transfer of fresh fish to the exposure cells, at least four days recovery should be allowed prior to using the fish for monitoring.

Replacement frequency of fish

Fresh fish should be introduced into the exposure cells every three weeks to overcome the possiblity of fish acclimation to the water.

Photoperiod

Continuous dimmed lighting should be maintained to damp the diurnal ventilatory rhythm of the fish and enable assessment of fish activity every 1 or 2 hours.

Fish feeding

The fish should be fed during the holding and acclimation periods but not during the monitoring. Largemouth bass may be fed live fish while rainbow trout will readily accept pelletized trout food.

Exposure water flow rate

A minimum flow rate of 0.5 L/min and a preferred flow rate of 0.75 to 1.0 L/min should be maintained to orient the fish and keep the exposure cells clean.

4.6.4 DATA ANALYSIS

Arrangement of test-fish

	MONITORING BANK		ACCLIMATING BANK
Time X	7 EXPERIMENTAL FISH	7 CONTROL FISH	14 ACCLIMATION FISH
Time X+1	7 EXPERIMENTAL FISH	7 CONTROL FISH	14 ACCLIMATION FISH
	FISH 1 to 14		FISH 15 to 28

General statistical approach

	MONITORING BANK	
Time X	EXPERIMENTAL	CONTROL
Time X+1	EXPERIMENTAL	CONTROL
	7 FISH	7 FISH

Two comparisons, with four possible outcomes, are of interest when using fish activity to monitor water quality.

1. Comparison of the activity of the experimental fish to previous activity; and

2. Comparison of experimental fish activity to control fish activity for the same time interval.

- If both comparisons are significantly different, then the experimental fish have altered their activity from normal and an alarm situation exists.

- If the experimental fish have altered their activity significantly between time periods but heir present activity is not different from that of the control fish, then check for evidence of extraneous disturbance.

- If the experimental fish not changed their activity but is is significantly different from control fish activity, then there is a problem in the control water supply.

- Finally, if neither comparison shows a significant difference, then continue monitoring.

Frequency of data assessment

Assess fish activity every hour or two. This is a sufficient duration to collect a representative estimate of respiratory rate for each fish and also frequent enough for remedial action and detection of short-term water quality changes.

Sample size for data assessment

Fifteen or twenty 1 minute counts per hour are sufficient to produce a representative estimate of the respiratory rate of each fish.

Statistical evaluation

1. A nonparametric test is suggested since observations are taken from the same subjects and therefore are not independent and also since available evidence indicates respiratory rate of fish is not normally distributed.

2. To compare the experimental fish activity at different time intervals use the Wilcoxon matched-pairs test (parametric equivalent is the paired T-test). This is a rank test which examines the direction and magnitude of fish respiratory rate change over time which is valid when taking repeated independent measurements from the same subjects.

3. The Mann Whitney "U" test may be used to compare the experimental and control fish at any one time interval. This test is very similar to the Wilcoxon matched-pairs test but is used to compare observations of different subjects. Its parametric equivalent is the independent T-test.

4. The advantage of these tests are:
   1) simplicity,
   2) statistical validity,
   3) lack of assumption regarding distribution of data,
   4) comparison of individual fish activity over time, and
   5) suitability for use when a control water source is unavailable (ie. use Wilcoxon matched-pairs test).

5. Time Series Analysis may be a useful tool for analyzing fish physiograph data. However, methods developed to date rely on prior knowledge of the time of introduction of a toxicant into the water and thus appears only suited to laboratory exposures. Dr. Ian McNeil of the University of Western Ontario is presently developing a time series approach called Intervention Analysis which may be used without prior knowledge of treatment initiation.

4.6.5 PHYSIOGRAPH SYSTEM COST ESTIMATES

The following cost estimates are for the physiograph only and do not reflect total costs associated with setting up a complete fish physiograph monitoring laboratory.

If a manual interpretative strip chart recording system is desired, the cost for this system would be approximately $8,100.00, as shown below. These costs are based on construction and purchasing in Canada.

MANUAL SYSTEM

Exposure cells (24) including electrodes	$2,500.00
Amplifier/filters (24) and power supply	$2,800.00
Signal carrier cable	$ 100.00
Two channel linear strip chart	$2,500.00
Exposure cell sound proof enclosure	$ 200.00
TOTAL	$8,100.00

The VPI and SU minicomputer system has been operated successfully by Dr. J. Cairns and Dr. D. Gruber. The total cost for their system is estimated to be $21,525.00.

However, there is evidence to suggest that a less costly microcomputer system would be suitable for interfacing with the manual system to provide complete automation. The software has not been developed for a microcomputer system however, Mr. D. Genner, Institute of Computer Science, University of Guelph advises that the software development would be approximately $2,500.00. The total microcomputer system cost is estimated to be $11,162.00. Cost breakdown for the mini and microcomputer systems is shown on the following page.

	Minicomputer System	Price	Microcomputer System	Price
Computer	DEC PDP 11/V03-L Model SRBX-SSB 32K	$12,500	North Star Horizon 780 Model 64K -2D	$ 7,062
Commercial software	DEC RT/11-36	$ 1,600	CP/M Operating System[2]	-
	Fortran (no listing)	$ 1,020	Microsoft Fortran[2]	-
Custom software	Fortran programs plus source listing documentation user guide	$ 3,200	To be developed	$ 2,500
A/D converter	32 channel and real time clock	$ 1,155	2,16 channel and 1 real time clock	$ 1,600
D/A converter	2 channel	$ 425	4 channel	-
Terminal/printer	Decwriter LA36	$ 1,625	Decwriter	-
	Total	$21,525		$11,162

[1]one time expenses
[2]included in computer package price

Furthermore, a maintenance contract would increase the total cost for the two systems. An annual maintenance contract for the DEC and North Star system are estimated to be $2,500 and $825, respectively.

4.7 CONCLUSIONS AND RECOMMENDATIONS

IEC investigated seven fish physiograph systems which had been used in a variety of laboratory research applications; none of which had been applied to drinking water supplies and none had been used in Canada. IEC's unique field application of fish physiography showed that this research technique could be used as a reliable field monitoring device. The physiograph developed by IEC essentially integrated and optimized the other researchers' systems for Canadian application. The system developed by IEC will facilitate the use of fish physiography by other scientists in their investigations. However, the study has shown that further research is necessary to computer automate the physiograph system.

IEC's research and literature review has shown that organic chemical concentrations required to elicit a respiratory response by fish were several orders of magnitude higher than organic concentrations found in the drinking water supply at Brantford. Fish did not alter their respiratory activity to change in levels of organics at Brantford. However, fish respiration could be used to monitor organic levels if ambient concentrations are significantly higher than levels in waters presently measured in the Grand River.

Dose-respone experiments using phenol, zinc and ammonia showed that alterations in respiratory parameters occurred within several hours at 10 - 50% of acutely lethal levels (96 h LC50). Cough and ventilation rate were sensitive respiratory parameters. In addition, ventilation amplitude illustrated acutely lethal levels and severe elevations in concentration. Different compounds evoked different respiratory responses on the fish. Phenol and zinc which act directly on the gill membrane affected cough and ventilation rate while ammonia which interferes with gill physiology did not stimulate a cough response but altered the pattern of ventilation.

IEC's research has shown that fish physiograph systems may be operated to monitor chemical slugs or spills at sub acute levels and for monitoring the toxicity of effluent discharges. Future research should consider automating the system with a computer for real time evaluation of the data.

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The following members of IEC contributed to the research:

Douglas G. Langley	- Project Principal
Jerry Fitchko	- Project Management
Douglas H. Stendahl	- Principal Biologist
Wayne C. Wager	- Project Biologist/Supervisor
Thomas W. Beak	- Project Advisor
John L. Robertson	- Analytical Supervisor
Paul Commarmond	- Graphics
Sheila Cox	- Stenographic

APPENDIX 1: BIOMONITORING OF ORGANIC COMPOUNDS IN WATER
(ORF REPORT 81-1)

1. INTRODUCTION

This report details the results and methodology used for the analysis of fish samples and water samples for specified organic chemicals.

Biomonitoring is becoming an essential part of hazard assessment of environmental contamination. Some authors have predicted that bio-accumulation potential for organic chemicals is readily estimated from consideration of their chemical structure[1]. Their main conclusion was that only highly lipophilic chemicals are likely to pose residue problems in fish, and consequently fish tissue should provide both a concentrated and integrated sample of such compounds. Therefore, multiclass chemical residue analysis of fish from major watersheds should offer a valuable means of detecting contamination of potentially hazardous chemicals.

For this study, ORF was supplied with fish meal, whole fish samples and water sample extracts (collected by passage of water through XAD-2 resin cartridges which were eluted with acetone) by IEC Limited. The supplied samples were from a biomonitoring study with the fish samples and water extracts being representative of various time periods during the study.

The analyses performed at ORF involved class selective type analyses for specific target compounds and involved tailored cleanup and analytical determinations for the compounds. These specific chemicals are conveniently divided into the following groups:

1. Polycyclic Aromatic Hydrocarbons (PAH), aromatic hydrocarbons.
2. Organochlorine pesticides (OCs), PCBs, chlorinated benzenes.
3. 2,4-D, 2,4,5-T acid herbicides.
4. Chlorinated phenolics (penta-, tetra- and tri-chloro').

5. Phthalate esters.
6. Organophosphorus pesticides and triazines.
7. Aliphatic and alicyclic organo halogen compounds e.g. chloroethanes, hexachlorobutadiene.
8. Aromatic organo-halogen e.g. chlorotoluenes, chlorostyrenes.

2. EXPERIMENTAL

A. Preparation of Sample Extract

1. Fish

The fish were supplied in frozen form and retained in a freezer compartment until ready for analysis. The fish, when semi-thawed, were scissored into a beaker and the cut up sample polytroned (using medium sized generator) until visibly homogenized. Aliquots were weighed immediately from the homogenate for tailored cleanup prior to specific chemical analysis.

2. Water

The water extract was made up of two fractions:

(a) A dissolved organic extract associated with the main resin body.

(b) A particulate organic extract associated with the retaining glass wool portion that was sitting on top of the resin column during the passage of water through the cartridge.

For both fractions the samples consisted of an acetone extract of the solid material viz., resin or glass wool. An additional extract of the solids was performed using acetone, followed by a final extraction with CH_2Cl_2. The drying and concentration of the extracts was performed in the following manner:

(i) Drying of Solvent Extraction

The flask containing the combined acetone/CH_2Cl_2 extracts was placed into a bath containing liquid nitrogen (LN_2). Flasks containing aliquots of acetone/CH_2Cl_2 were also chilled in LN_2 for rinsing purposes. Upon removal from the bath the dried extract was quickly transferred into a Kuderna-Danish (KD) evaporator. This was achieved by pouring the extract through a small volume of Na_2SO_4 seated on a glass wool plug placed in a glass funnel. The ice left behind was washed with small aliquants of the chilled solvents which were added to the KD flask.

(ii) Concentration

The KD flask was fitted with a three-chamber Snyder column and the extract reduced to a small volume (1 - 2 mℓ) by gentle heating in a water bath. The Boiling of the solvent was aided by addition of porcelain boiling chips. The evaporator was removed from the heat source when the level in the KD thimble was definitely less than 1 mℓ. Rapid cooling of the evaporator occurs upon removal from the heat source and the condensing solvent washes the walls of the KD. For final concentration to a specific volume (1 mℓ) the solvent was transferred from the KD to a small scale K-D evaporative concentrator (Kontes K-569350). It consists of a small scale graduated concentrator with a modified micro-Snyder column. The final evaporation was achieved with a Kontes tube heater (K-720000). After removal of the Kontes tube from the apparatus, it was capped with a ground glass stopper and refrigerated prior to GC analysis.

B. Extraction and Cleanup

1. Fish

(a) Groups 2, 5, 7 and 8

About 2.5 g (accurately weighed) sample is ground with ∿50 g Na_2SO_4 (until free-flowing) and Soxhlet-extracted (precleaned glass thimbles) for 5 hours with 25% ether in hexane. The extract is made to 150 mℓ volume with hexane and 15 mℓ of this is used for lipid determination (concentrated and weighed). The remaining portion of the extract is concentrated to ∿5 mℓ and cleaned up on 2% water-deactivated Florisil (regular grade which had been heated to 300°C and then deactivated with 2% water).

A chromatographic glass column was packed with Florisil (30 g is poured into the column containing about 70 mℓ hexane, topped with 1 cm sodium sulphate and the hexane drained to the top of the Na_2SO_4) and the sample extract (maximum 0.5 g oil or fat) was added and three fractions were eluted successively at approximately 5 mℓ/minute as follows:

Fraction I: eluted with about 180 mℓ hexane (predetermined by preliminary test elution to get ∿10% of p,p-DDT into hexane fraction - this ensures all DDE into hexane fraction), contained DDE, PCB, chlorobenzenes, mirex, photomirex, aliphatic, alicyclic and aromatic organo-halogen compounds.

Fraction II: eluted with 300 mℓ of 30% dichloromethane in hexane, contained DDT, TDE, dieldrin, HE, chlordanes.

Fraction III: eluted with 250 mℓ dichloromethane containing phthalate esters.

Suitable aliquots from the different fractions were taken for GC-EC and/or GC-FID analysis.

(b) Group 1

Fish homogenate (5 g) was placed in a flask (RB;250 mℓ) to which was added methanolic KOH (2N;30 mℓ) and the mixture heated to refluxing for 3 hours. The material was then transferred while still warm to a separatory flask (200 mℓ capacity) with the aid of rinsings using MeOH:H_2O (4:1;40 mℓ). Cyclohexane (80 mℓ) was added to the separator which was shaken for 3 minutes. The layers were allowed to separate and the MeOH:H_2O layer drained into a second separatory flask and the cyclohexane (80 mℓ) extraction repeated. The MeOH:H_2O layer was then discarded. The cyclohexane layers remaining in the two flasks were washed with MeOH:H_2O (1:1;40 mℓ) and then with water (40 mℓ). The combined cyclohexane layers were concentrated to near dryness on a rotary evaporator (reduced pressure/40°C) and any residue taken up in methanol (5 mℓ). A suitable aliquot was taken for HPLC analysis.

(c) Groups 3 and 4

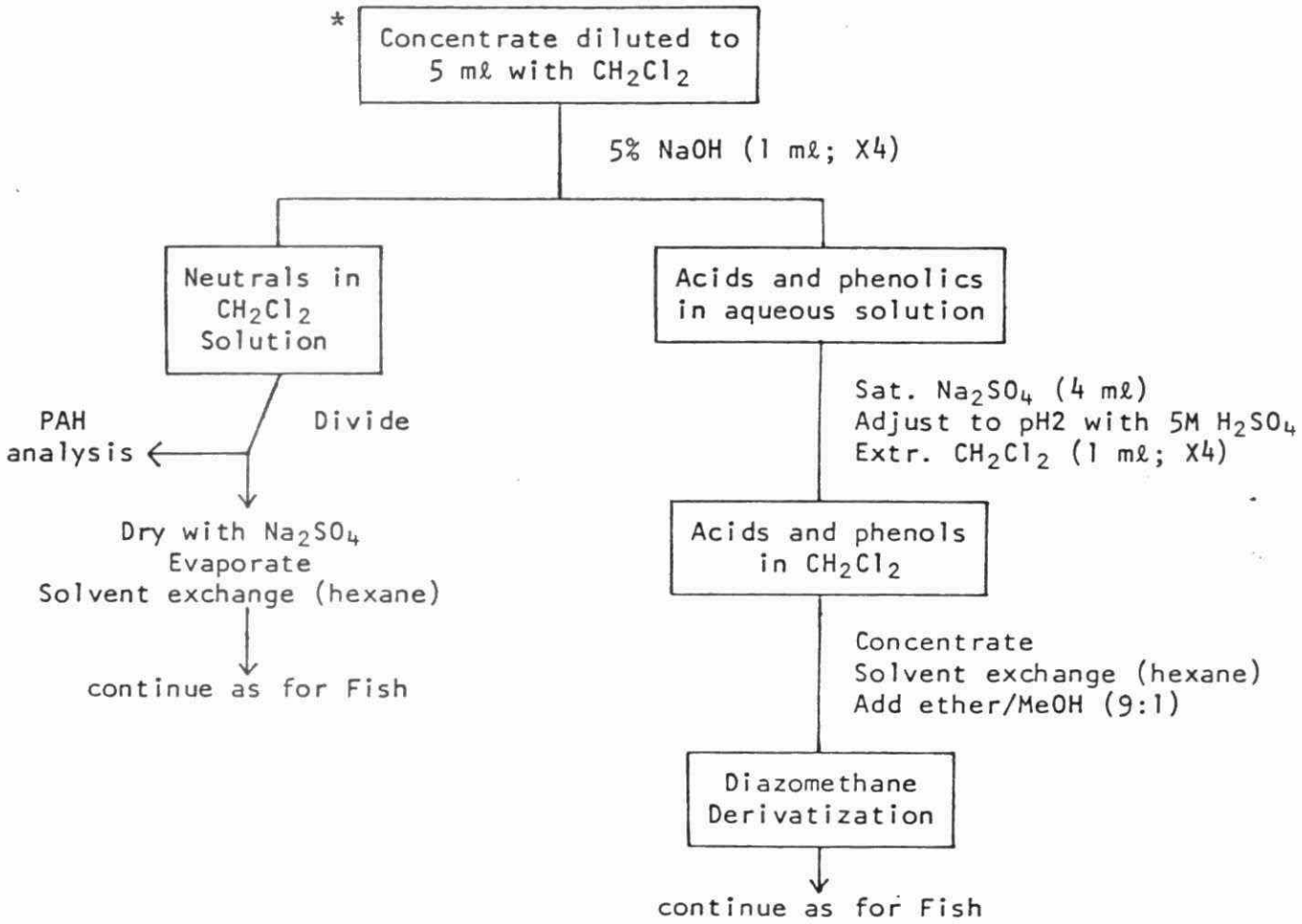
Fish homogenate (3 g) was Polytron blended with methanol:ammonia (100 mℓ:2 mℓ). The blend was immediately filtered (use fast filter paper) into a RB flask (250 mℓ), rinsed with additional methanol and concentrated to approximately a 10 mℓ volume. The concentrate was transferred to a separatory funnel with KOH (50 mℓ; 0.1N). The solution was extracted with CH_2Cl_2 (30 mℓ;X 2) and the CH_2Cl_2 discarded. The solution was acidified to pH2 (10% H_2SO_4) and extracted with CH_2Cl_2 (30 mℓ;X 2). The extract was concentrated to incipient dryness and esterified with diazomethane (3 mℓ). The solution was then cleaned up on Florisil by elution with 40% ether/hexane (250 mℓ). The eluate was concentrated to approximately a 5 mℓ volume, and made up to exactly 10 mℓ with benzene in a volumetric flask. A suitable aliquot was taken for capillary GC/EC analysis.

(d) Group 6

Fish homogenate (3 g) was blended with CH_3CN (50 mℓ) and filtered through glass wool into a separatory funnel (500 mℓ), rinsing with an additional volume of CH_3CN (25 mℓ). The extract was extracted with hexane saturated with CH_3CN (75 mℓ; X 2) and the hexane discarded. Water (300 mℓ) and saturated NaCl (25 mℓ) was added to the separatory funnel. The resulting solution was extracted with CH_2Cl_2 (75 mℓ;X 3). The extract was dried (Na_2SO_4), concentrated to near dryness, and taken up in benzene (5 mℓ). A suitable aliquot was taken for GC/N-P chromatographic analysis.

2. Water Extracts

A separation of the extracts into acidic and neutral fractions was achieved using classical chemical separations as detailed in the schematic outlined below.



* For the analysis of Group 6 compounds the extract was used directly without any chemical separation procedures being performed.

The above procedure was followed for both the raw water extract (resin) and particulate extract (glass wool). Procedural blanks were carried out at the same time as sample workup.

C. Analytical Instrumentation

1. Gas Chromatography

The gas chromatographs used were:

(a) A Varian Model 3700. Digital integration of the electrometer output was achieved with the use of a Spectra Physics SP4020 data interface and a SP4060 remote terminal connected to a Spectra Physics SP4000 central processor. The following GC parameters were used for analysis of group 7 and 8 compounds:

(i) Column: Glass (2 m x 2 mm) containing mixture 3% OV101/5% OV210 on Chromosorb W (80/100; H.P.)

Column Temperature: Isothermal 100°C for HCE and HCBut
Isothermal 180°C for OCS

Injector Temperature: 280°C
Detector Temperature: 300°C
Flow rate: N_2 ~40 mℓ/min
Sample size: 5 µℓ
Detector: Linearized Ni^{63} ECD

(ii) Column: Glass (2m x 2 mm) containing 5% OV101 on Chromosorb G.

Column Temperature: Programmed from 70°C - 280°C at 15°C/min for phthalate ester determination.

(Other parameters as above)

(b) A Hewlett Packard 5710A with a Spectra Physics SP4100 computing integrator; a Model 5709A Ni^{63} ECD; a Model 7672A Autosampler and a Model 18740A capillary column inlet and control.

Column: 12.5 m x 0.2 mm fused silica
Packing: SP2100 as supplied by Hewlett Packard
Injector Temperature: 250°C
Detector Temperature: 350°C
Column Temperature: 70°C - 220°C @ 8°C/min
Detector: ECD
Flow rate: ~ 1 mℓ/min

This instrument was used for the analysis of the Groups 2, 3 and 4 compounds.

(c) A Hewlett Packard 5700 GC equipped with a dual N-P detector. The following GC parameters were used:

Column:	2 m x 4 mm glass
Packing:	2% BDS on Chromosorb G (HP; 60/80 mesh)
Column Temperature:	185°C
Injector Temperature:	220°C
Detector Temperature:	300°C
Flow rate:	He ≈ 30 mℓ/min

This instrument was used for the analysis of Group 6 compounds.

2. High Performance Liquid Chromatography (Analysis Group 1 Compounds)

The HPLC used was a Beckman-Altex Model 322 with microprocessor.

Column:	25 cm x 4.6 mm
Packing:	Reverse Phase C_{18} ODS Ultrasphere
Mobile phase:	Isochratic 82% CH_3CN in water
Flow rate:	0.8 mℓ/min
Temperature:	ambient
Detector:	Fluorescence λ ex 280 nm λ ex 389 nm
Range/Sensitivity/ Time constant:	as required
Sample size:	50 µℓ

3. Gas Chromatography - Mass Spectrometry (GC-MS)

The GC-MS instrument used was a Hewlett-Packard 5992B, equipped with a Hewlett Packard desk top computor (9825A) with a flexible disk system (9885). The following GC-MS parameters were used for analysis:

Column:	2m x 2 mm Glass
Packing:	5% OV-101 on Chromosorb G (H.P.; 100/120 mesh)
Injector Temperature:	250°C
Column Temperature:	100°C to 250°C at 10°C/min
Flow rates:	He ~25 mℓ/min
Mass Spectrometer Source:	Electron bombardment

The GC-MS was operated exclusively in selected ion monitoring mode to search for specific compounds e.g. phthalates (m/e 149), naphthalene (m/e 128) and anthracene (m/e 178).

APPENDIX TABLE 1: RECOVERY STUDIES ON FISH SAMPLES[a]

Compounds Investigated	Fortification[b]		Minimum[c] Detectable Concentration (ppm)
	Spiking Level (ppm)	% Recovered	
OC Pesticides:			
Aldrin	0.008 0.08	93, 90 (92) 97, 95 (96)	0.002
Heptachlor	0.008 0.08	73, 87 (80) 90, 86 (88)	0.002
Lindane (γ-HCH)	0.0064 0.064	100, 109 (105) 100, 100 (100)	0.002
p,p´-DDE	0.016 0.160	92, 91 (92) 101, 95 (103)	0.002
p,p´-TDE (DDD)	0.024 0.24	94, 107 (101) 103, 98 (101)	0.004
p,p´-DDT	0.04 0.40	92, 103 (98) 88, 82 (85)	0.004
HE	0.0112 0.112	95, 93 (94) 95, 92 (94)	0.002
Dieldrin	0.016 0.16	102, 105 (104) 104, 100 (102)	0.002
Mirex	0.04 0.40	86, 85 (86) 88, 86 (87)	0.002
PCB:			
Aroclor 1254/1260 (1:1)	0.4 4.0	94, 91 (93) 78, 88 (83)	0.01
Chlorobenzenes:			
1,3,5	0.01 0.10	96, 97 (97) 97, 92 (95)	0.002
1,2,4	0.01 0.10	94, 96 (95) 96, 91 (94)	0.002
1,2,3	0.01 0.10	92, 95 (94) 96, 94 (95)	0.002
1,2,3,5/1,2,4,5	0.01 0.10	96, 99 (98) 98, 99 (99)	0.001
1,2,3,4	0.005 0.05	91, 95 (93) 98, 104 (101)	0.001
penta	0.003 0.03	97, 97 (97) 100, 111 (106)	0.001
hexa	0.003 0.03	102, 110 (106) 107, 114 (111)	0.001
Chlorophenols:			
2,3,4,6/2,3,5,6	0.33	88, 92 (90)	0.07
2,3,4,5	0.33	86, 89 (88)	0.03
penta	0.33	72, 73 (73)	0.03
Phenoxy Acid Herbicides:			
2,4-D	1.7	72, 92 (82)	0.17
2,4,5-T	0.33	113, 106 (110)	0.03
2,4,5-TP (Silvex)	0.33	111, 91 (101)	0.03
Organophosphates:			
Diazinon	-	-	0.01
Parathion	-	-	0.02
Malathion	-	-	0.06
Fenitrothion	0.16	81, 76, 71 (76)	0.02
Fenitro-oxon	0.17	128, 120, 110 (119)	0.04
Triazines:			
Atrazine	1.48	80, 80, 72 (77)	0.10
Simazine	1.78	83, 80, 75 (79)	0.06

APPENDIX TABLE 1: FOOTNOTES

(a) For these recoveries, capillary GC/EC was used except for OPs and Triazines which were analyzed by packed column GC/N-P. Duplicate 2.5 g aliquots of control fish were spiked and analyzed.

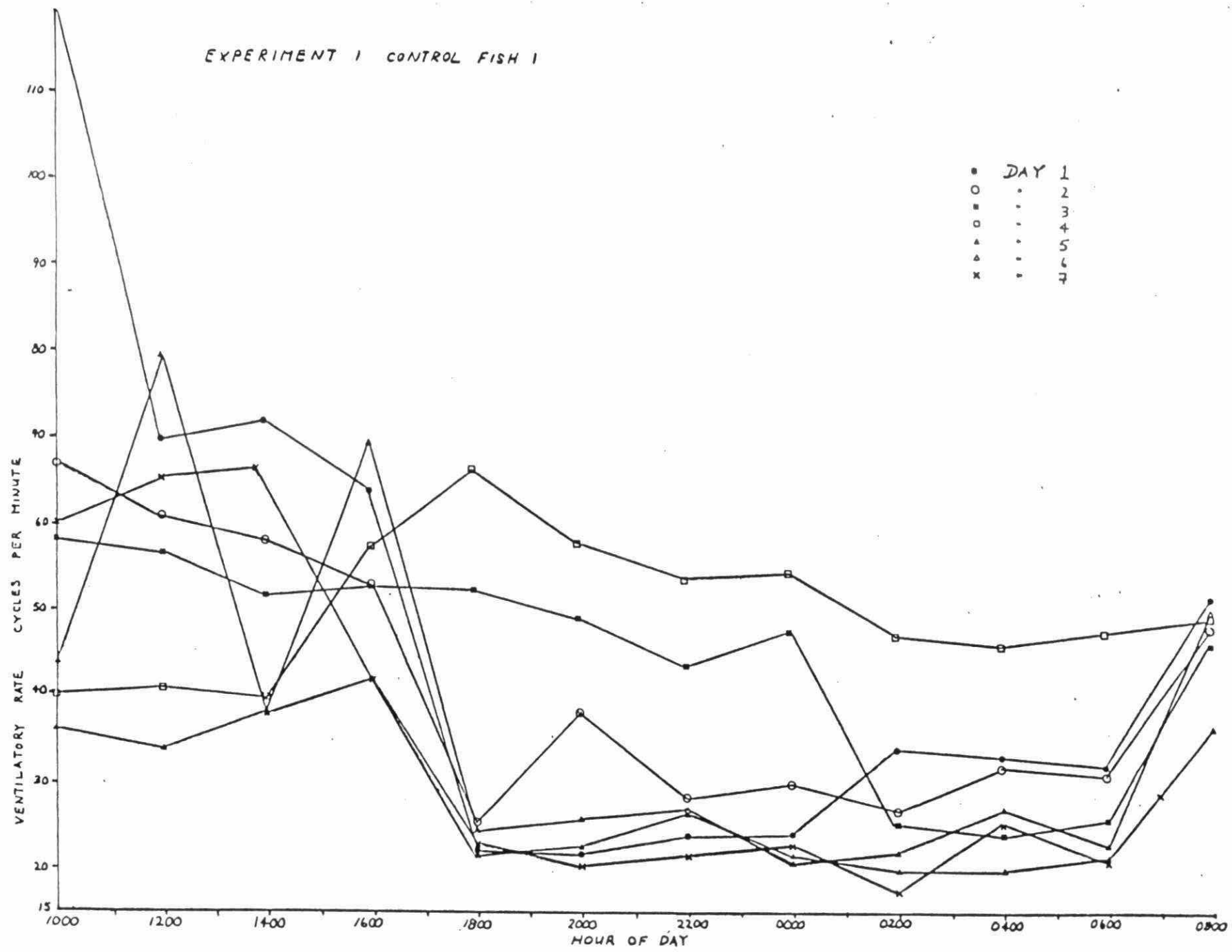
(b) Replicate values are shown separated by commas, while the mean values are given in the brackets.

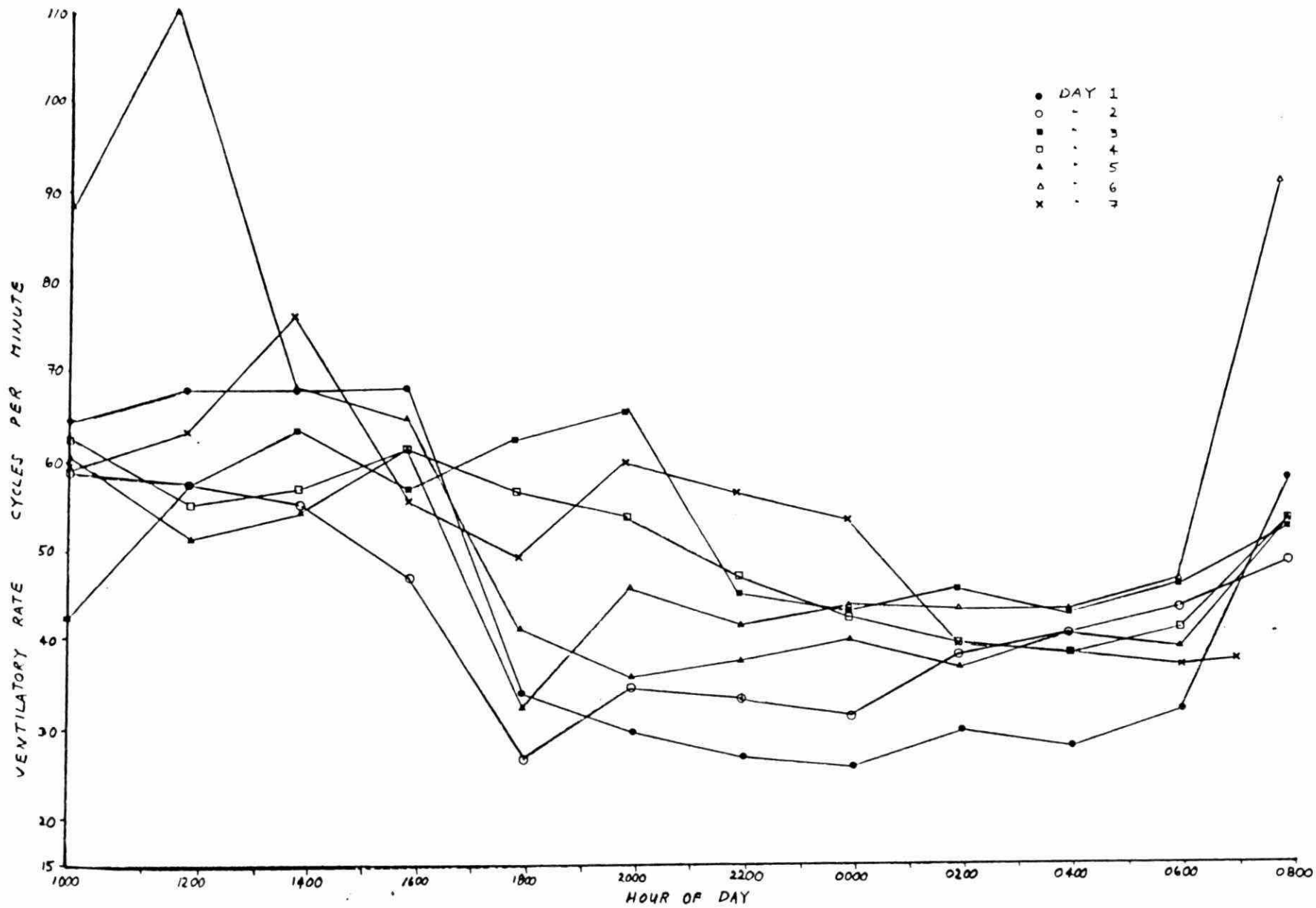
(c) These are based on 2.5 g aliquot fish and recorder peak height response of 1 cm (~ 2 x noise level) when 3 µL injected from a 10 mL volume. Levels below these would not be detected. For practical purposes, values lower than 0.01 ppm for OCs are not reported since confirmation is very difficult (if not impossible) at such levels.

APPENDIX TABLE 2: RECOVERY STUDIES ON SPIKED FISH

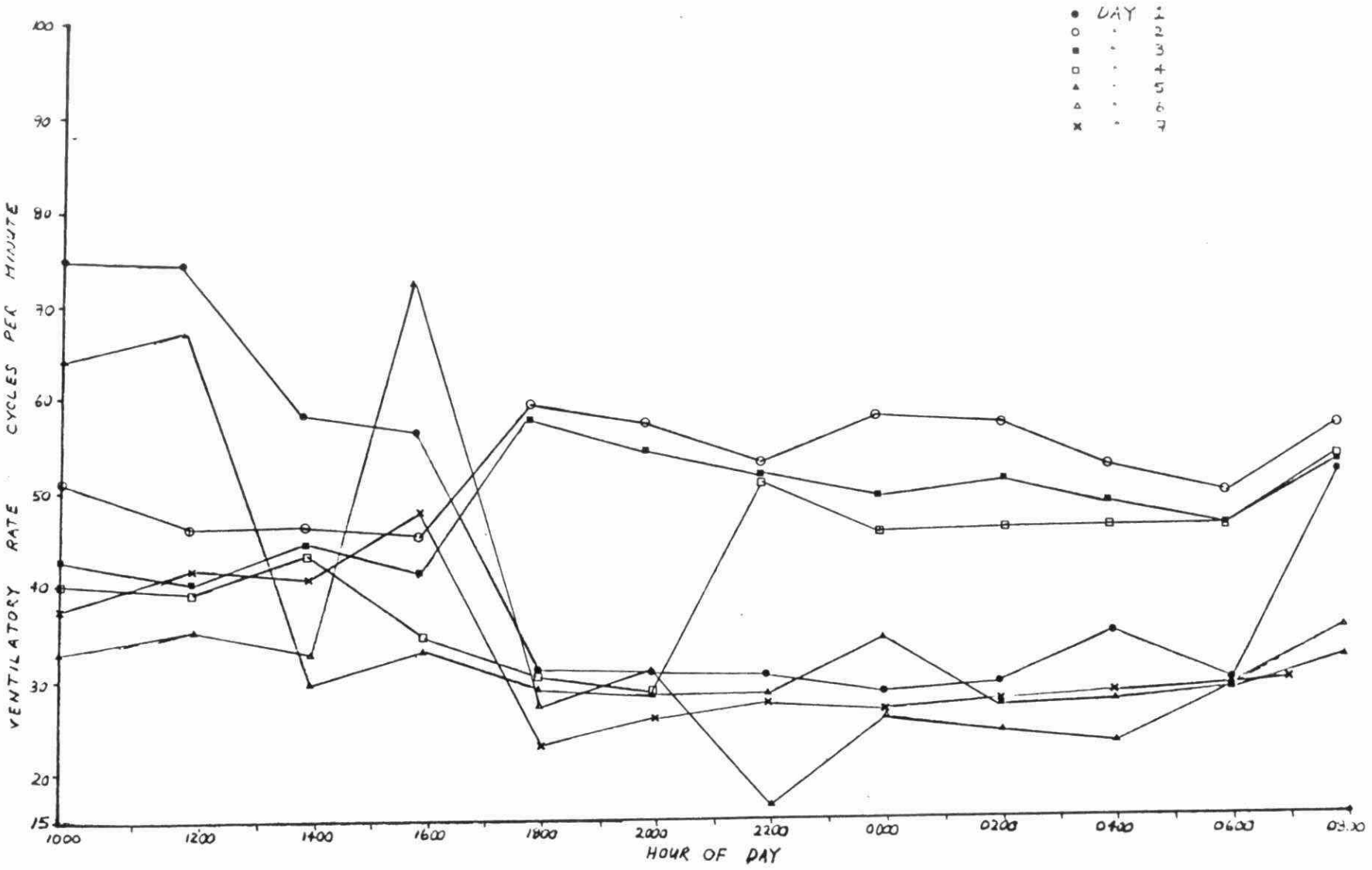
Compounds Investigated	Fortification		Minimum Detectable Concentration (ppm)
	Spiking Level (ppm)	% Recovered	
Benzo(a)pyrene	0.002	98, 108 (107)	0.001 [a]
	0.025	108, 103 (103)	0.001
Octachlorostyrene	0.014	100, 100 (100)	0.002 [b]
	0.14	98, 94 (96)	0.002
Diethylphthalate	0.20	87, 93 (90)	0.18 [c]
	2.00	94, 94 (94)	
Dibutyl phthalate	0.20	92, 94 (93)	0.18 [c]
	2.00	95, 93 (94)	
Dioctyl phthalate	0.25	87, 93 (90)	0.2 [c]
	2.50	90, 94 (92)	

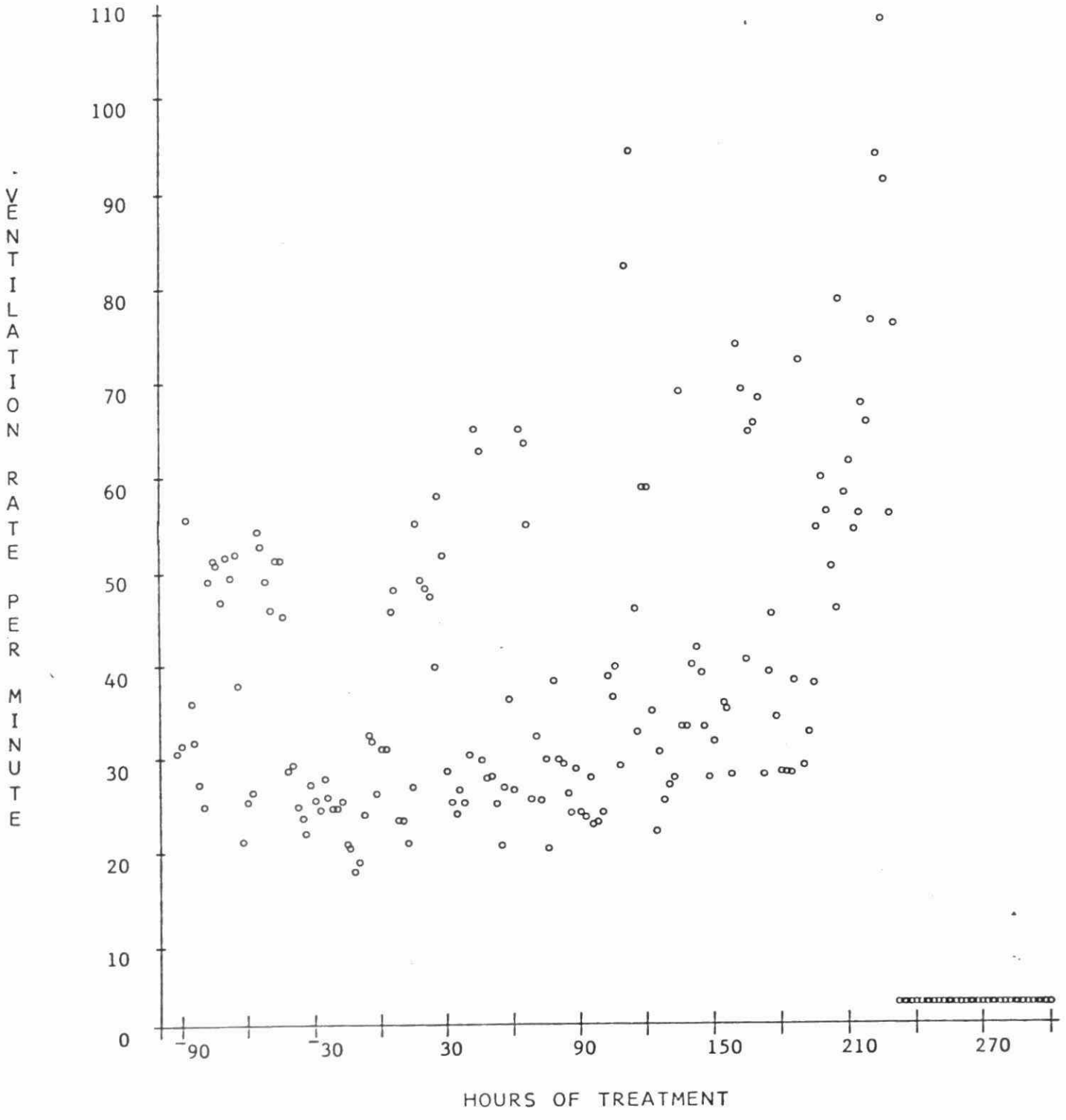
(a) based on fish aliquot (5 g); final volume (5 mℓ); injection volume 50 µℓ
(b) based on fish aliquot (2.5 g); final volume (10 mℓ); injection volume (5 µℓ)
(c) based on fish aliquot (2.5 g); final volume (5 mℓ); injection volume (5 µℓ)


EXPERIMENT 1 CONTROL FISH 1
DAY 1
2
3
4
5
6
7
VENTILATORY RATE CYCLES PER MINUTE
110
100
90
80
70
60
50
40
30
20
15
1000
1200
1400
1600
1800
2000
2200
0000
0200
0400
0600
0800
HOUR OF DAY

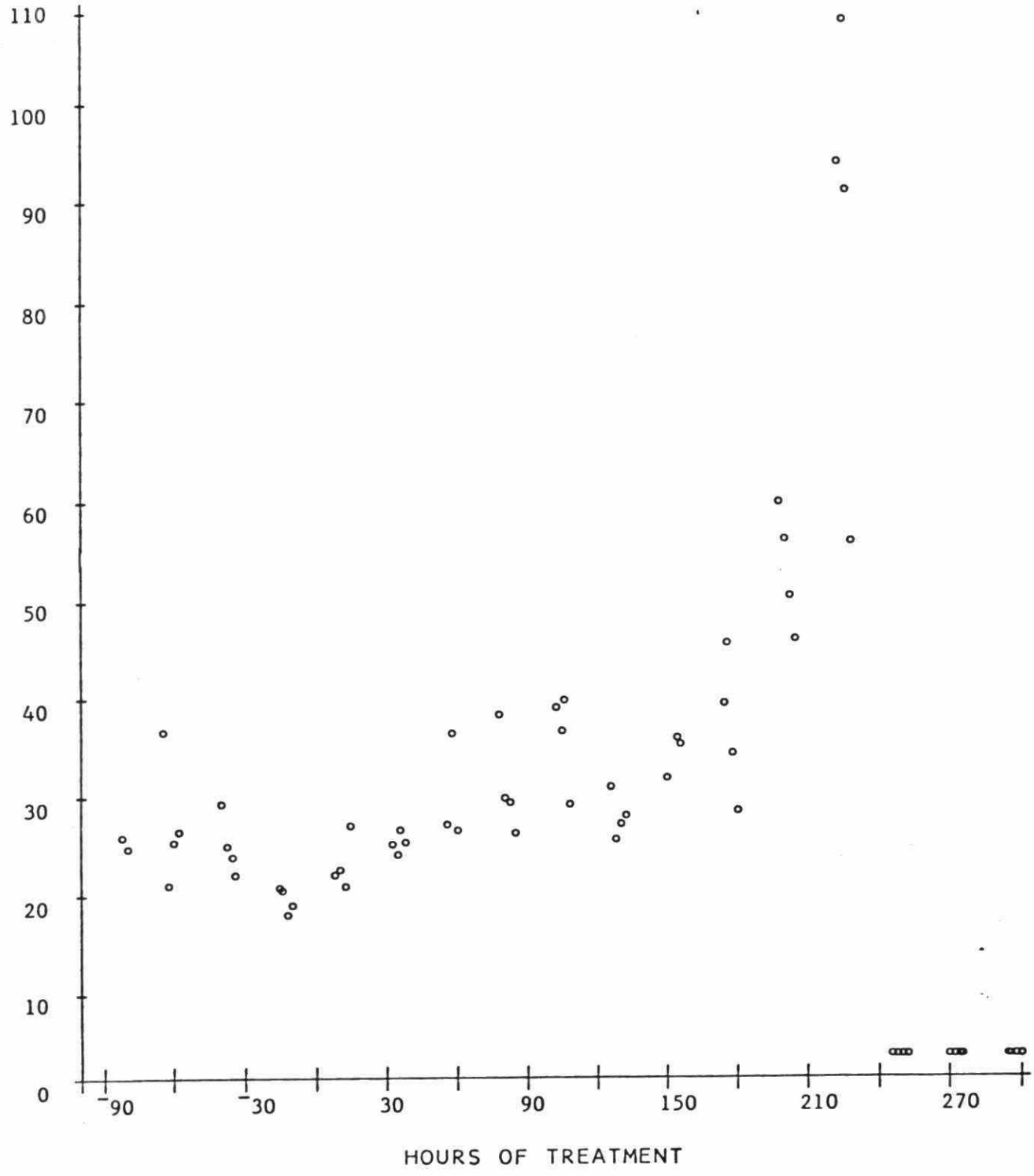

EXPERIMENT 1 CONTROL FISH 2
VENTILATORY RATE CYCLES PER MINUTE
110
100
90
80
70
60
50
40
30
20
15
1000
1200
1400
1600
1800
2000
2200
0000
0200
0400
0600
0800
HOUR OF DAY
DAY 1
2
3
4
5
6
7

EXPERIMENT 1 EXPERIMENTAL FISH 1

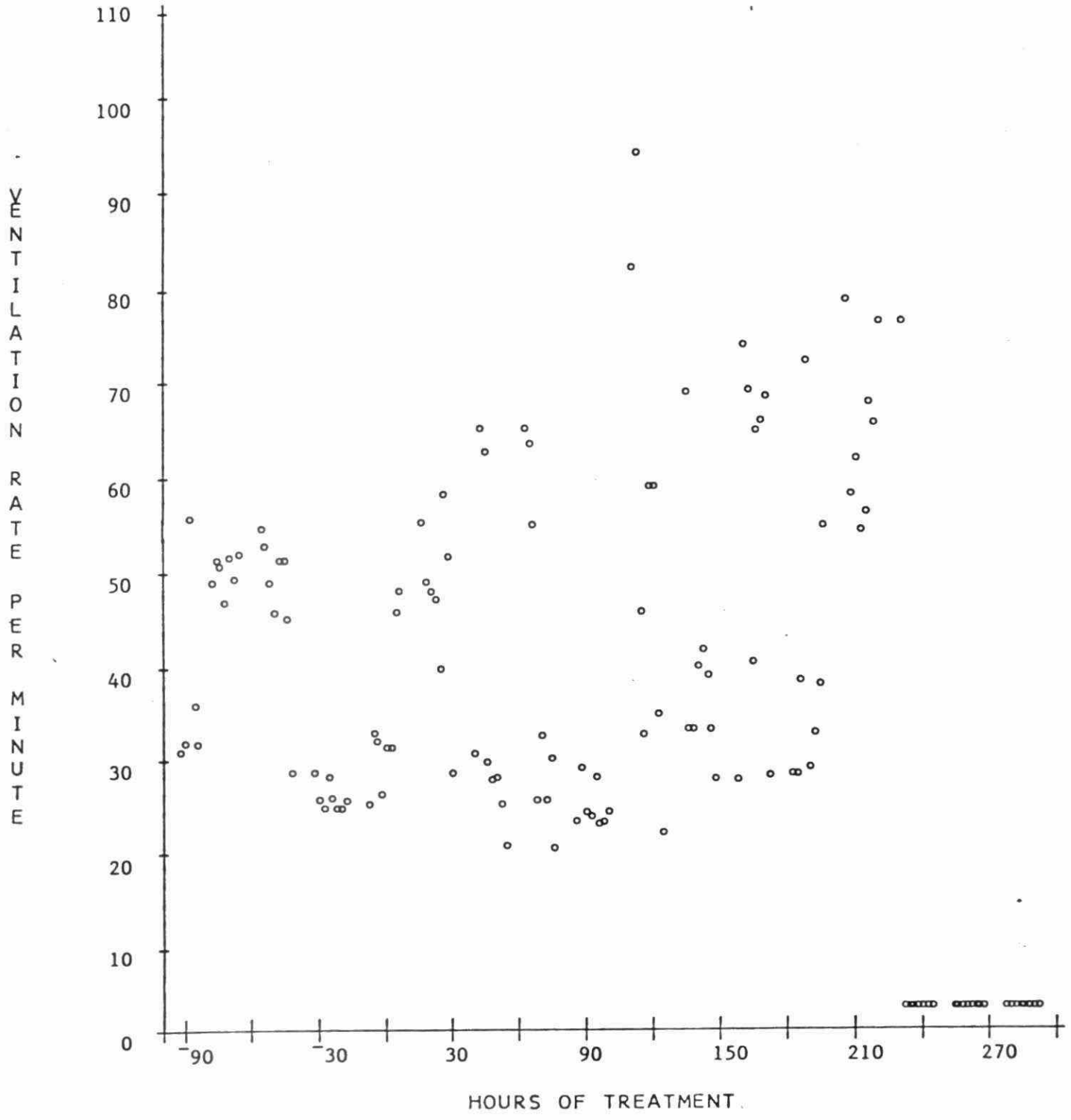




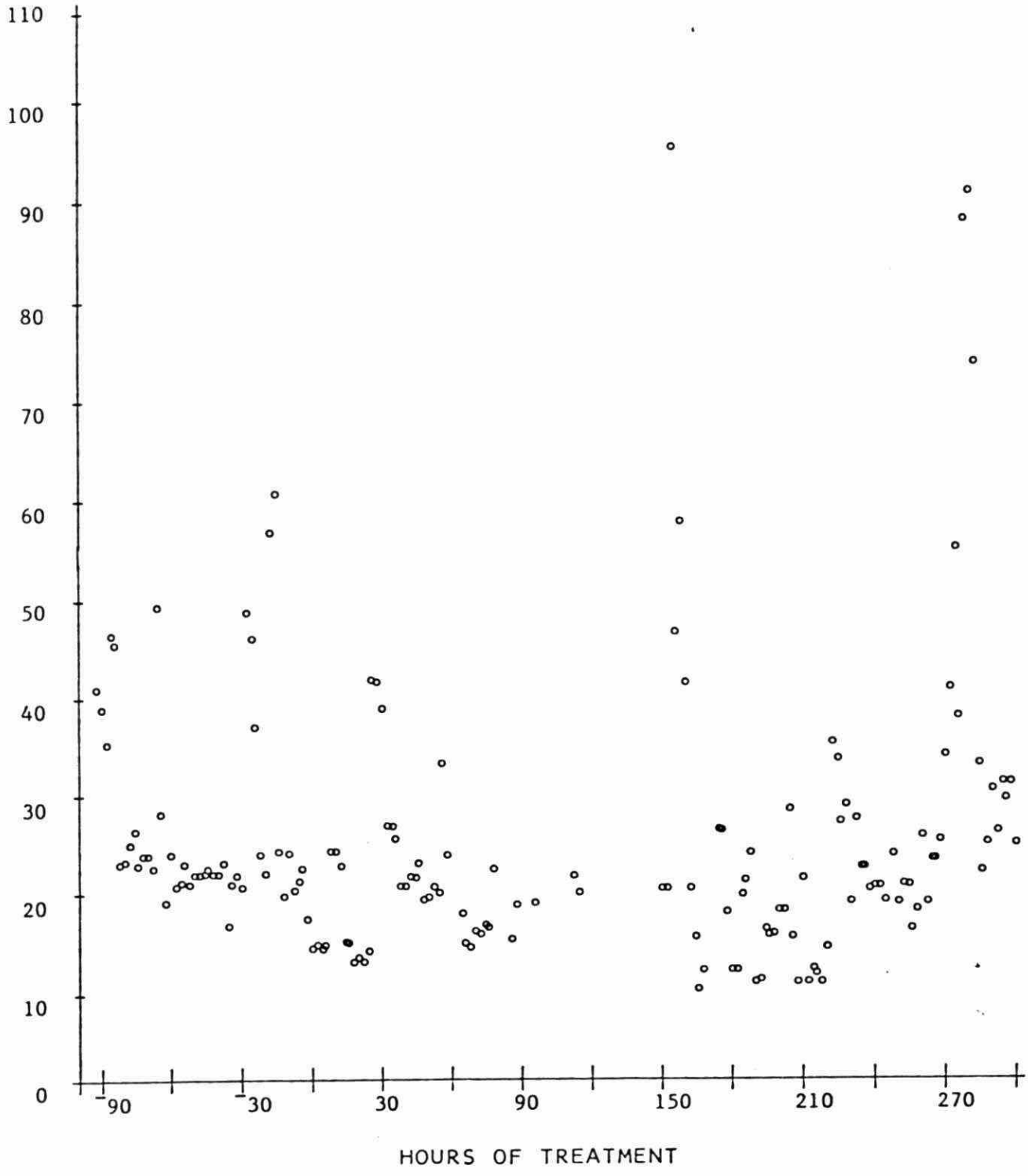
VENTILATION RATE EVERY 2 HOURS FOR FISH 1



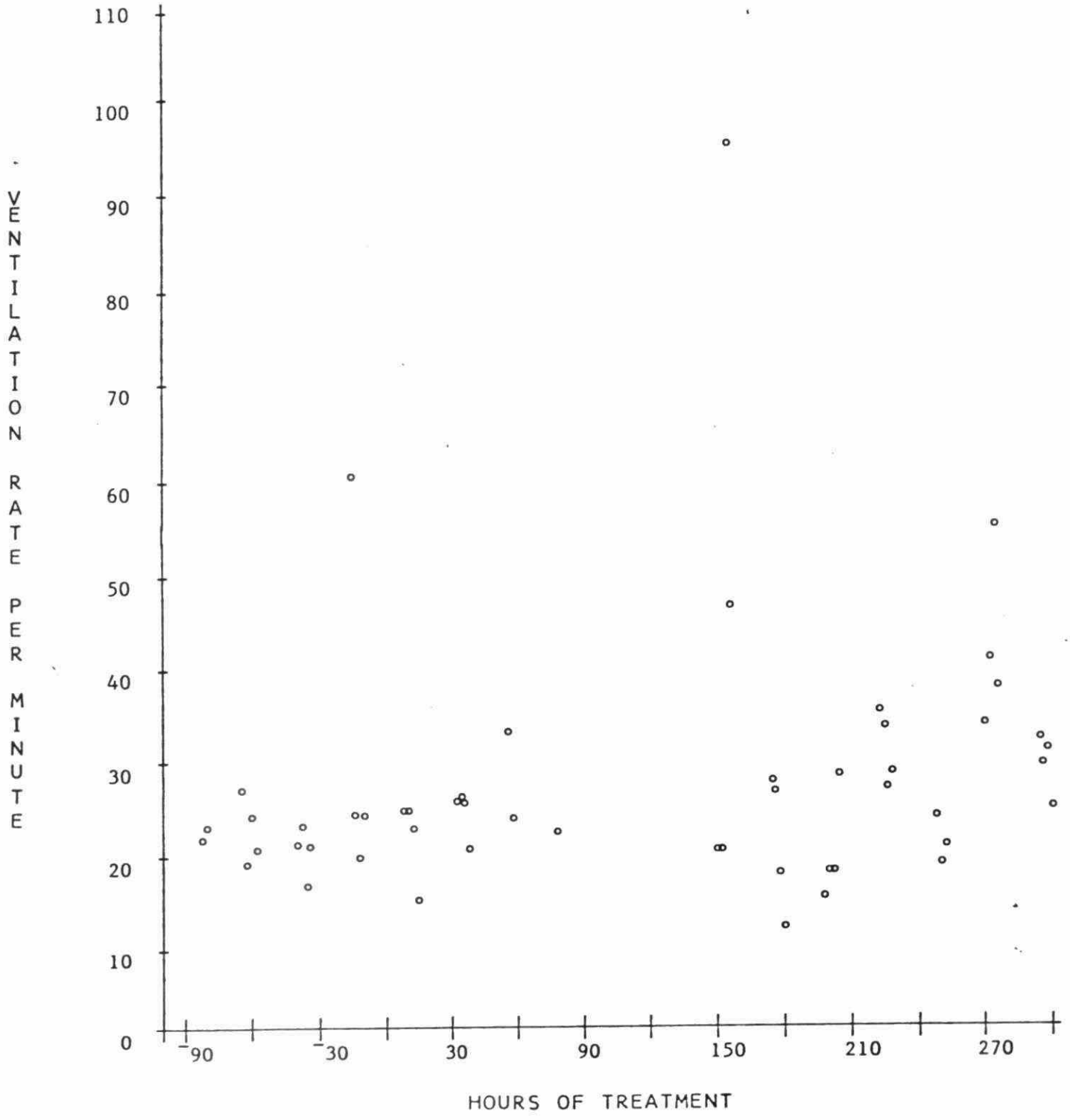
DAY VENTILATION RATE EVERY 2 HOURS FOR FISH 1



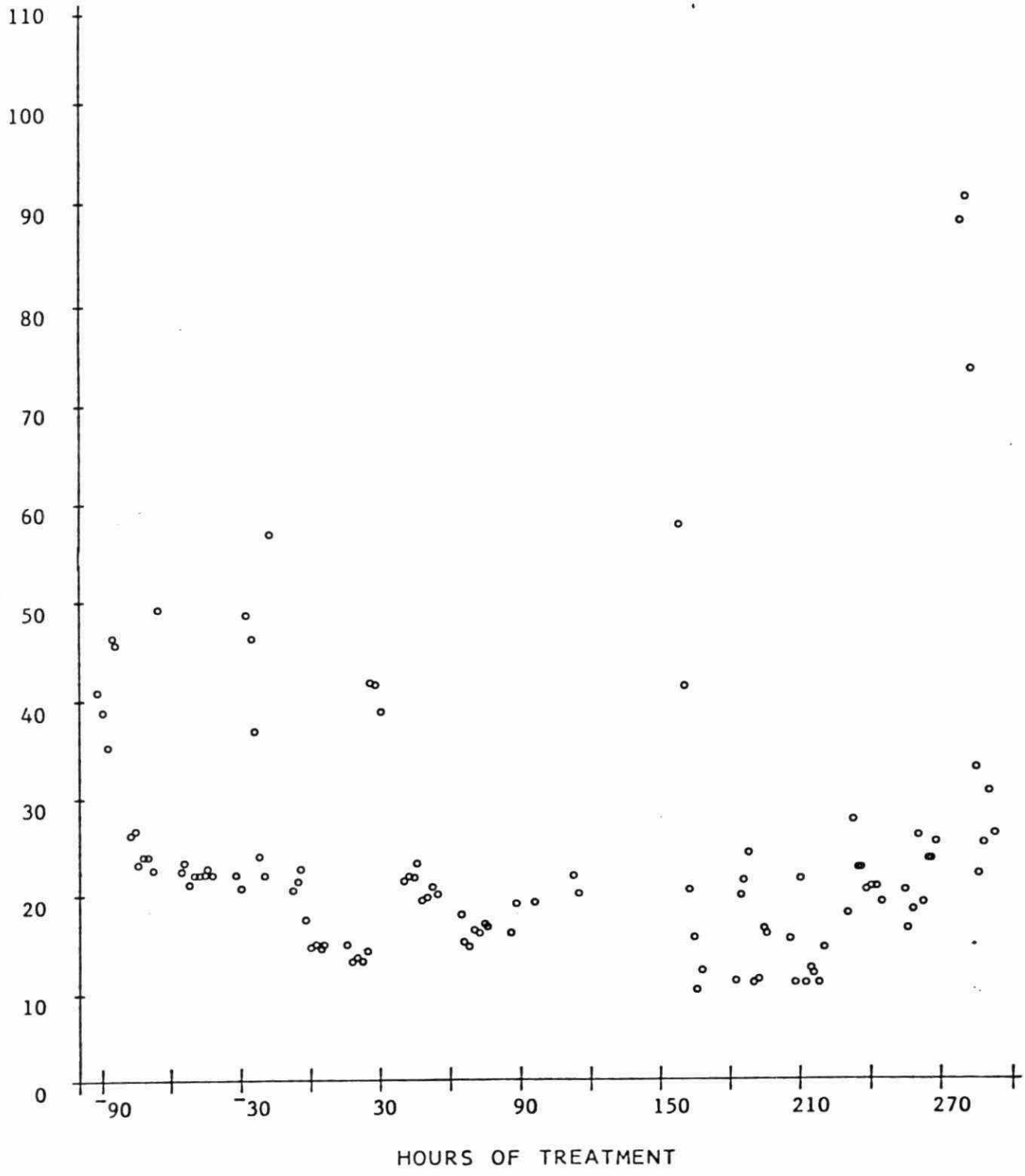
NIGHT VENTILATION RATE EVERY 2 HOURS FOR FISH 1



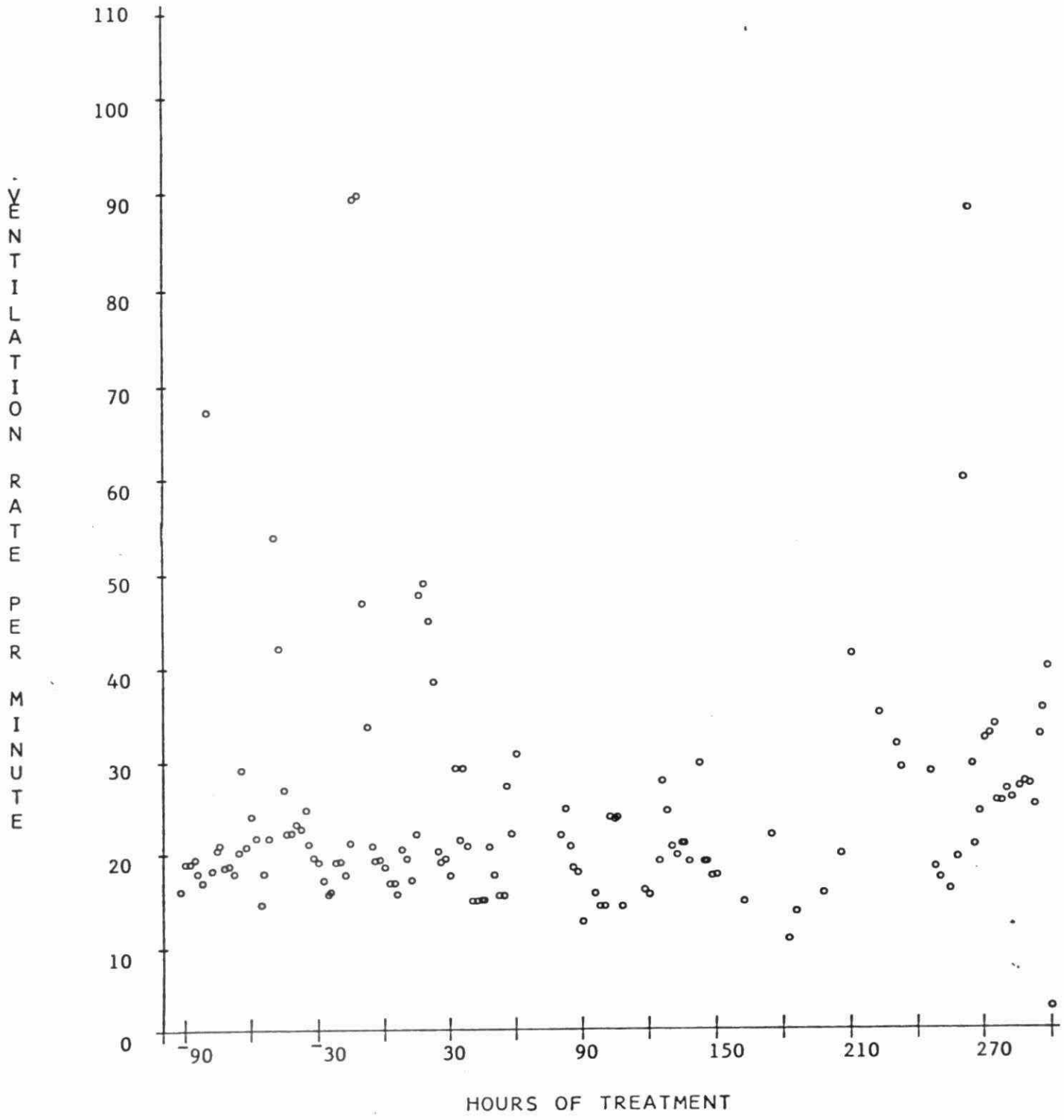
VENTILATION RATE EVERY 2 HOURS FOR FISH 2



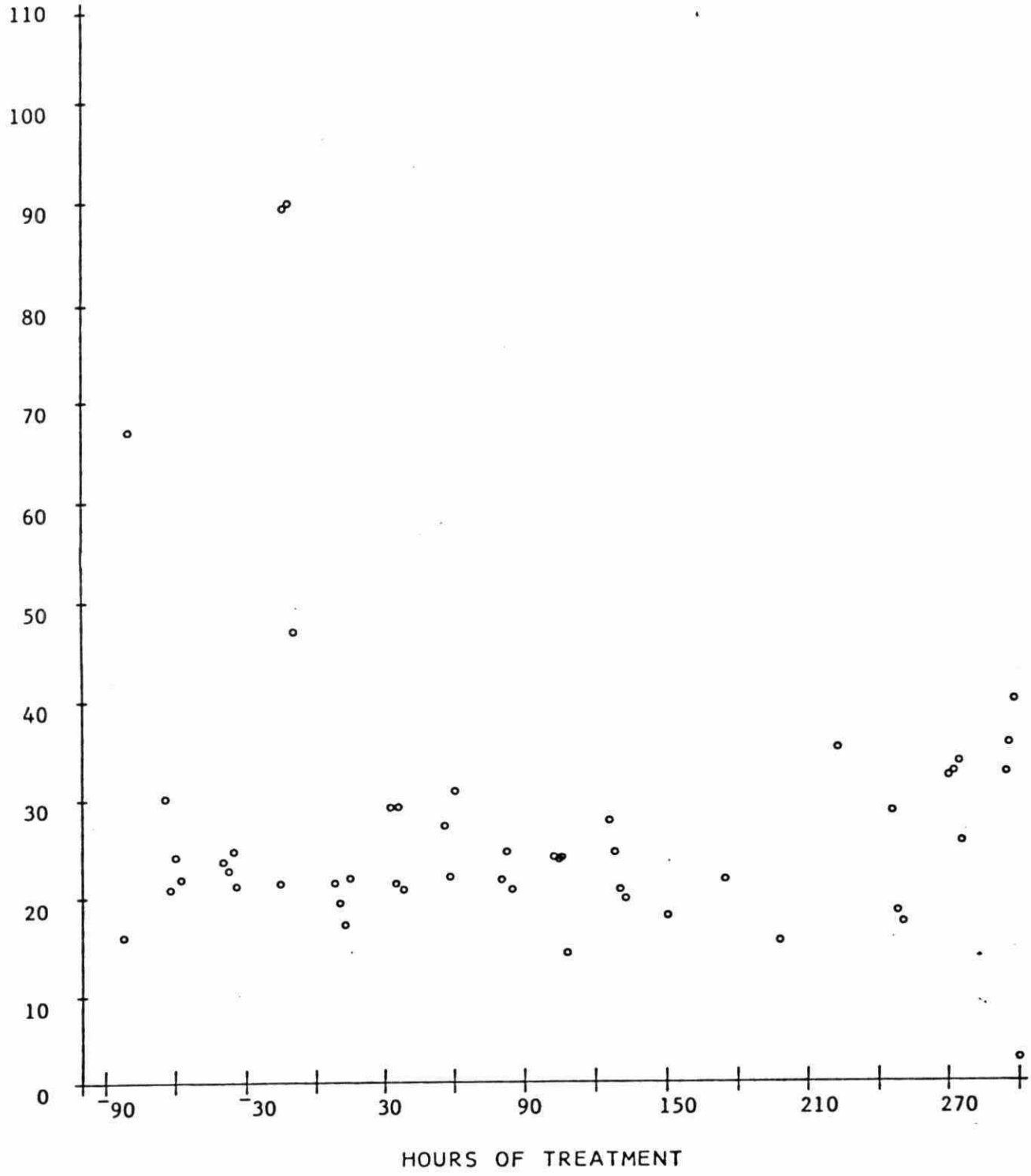
DAY VENTILATION RATE EVERY 2 HOURS FOR FISH 2



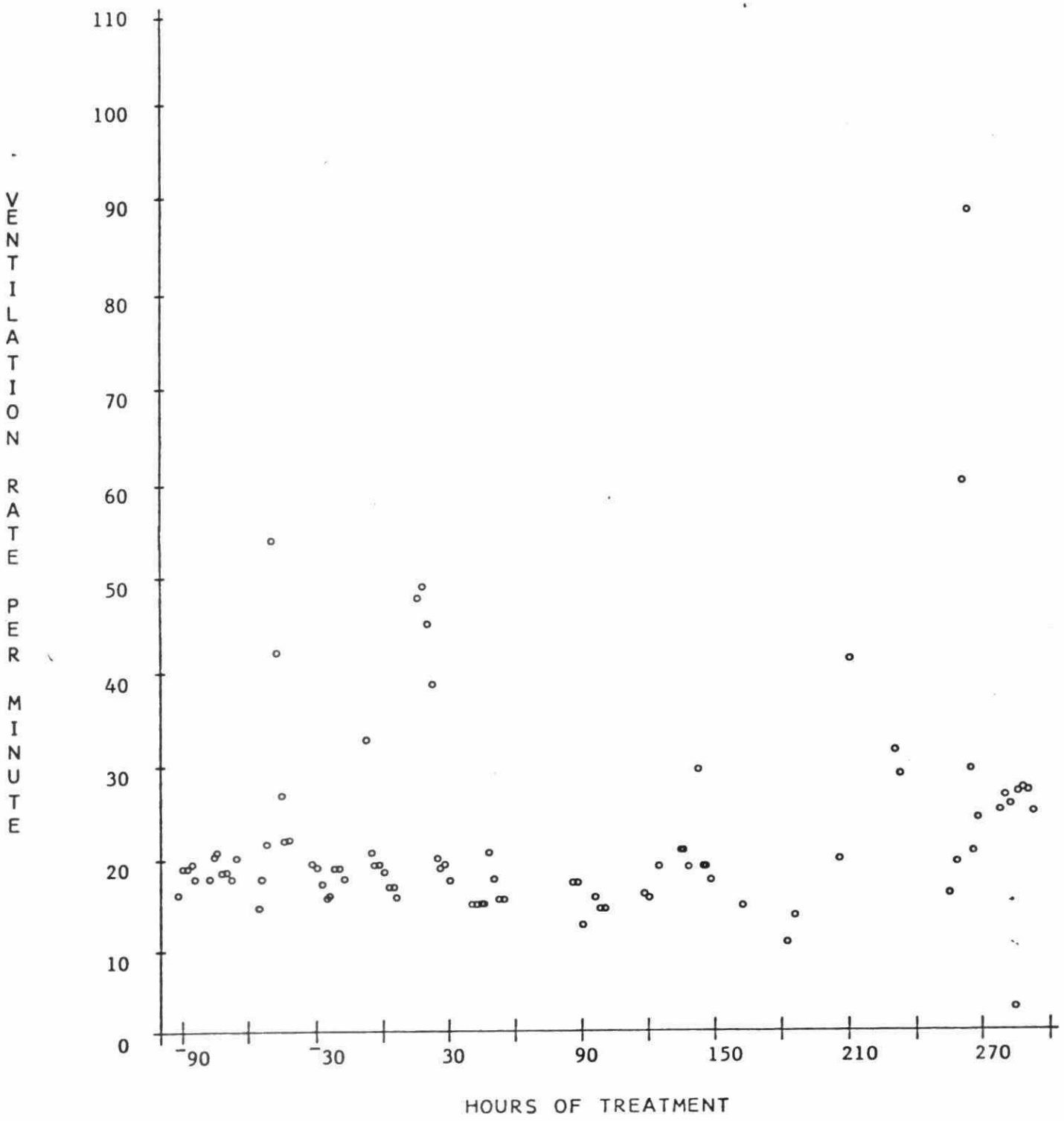
NIGHT VENTILATION RATE EVERY 2 HOURS FOR FISH 2



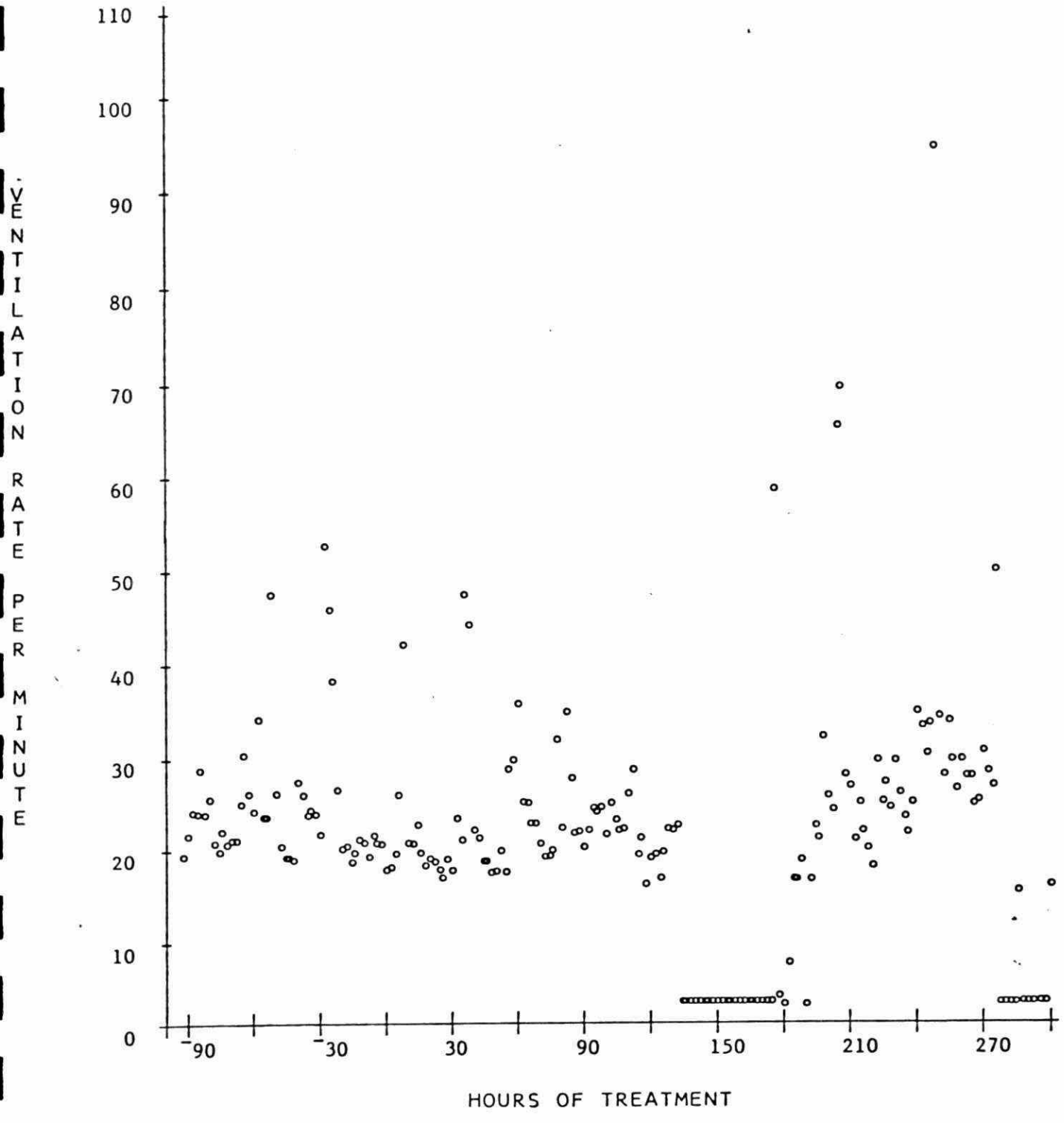
VENTILATION RATE EVERY 2 HOURS FOR FISH 3



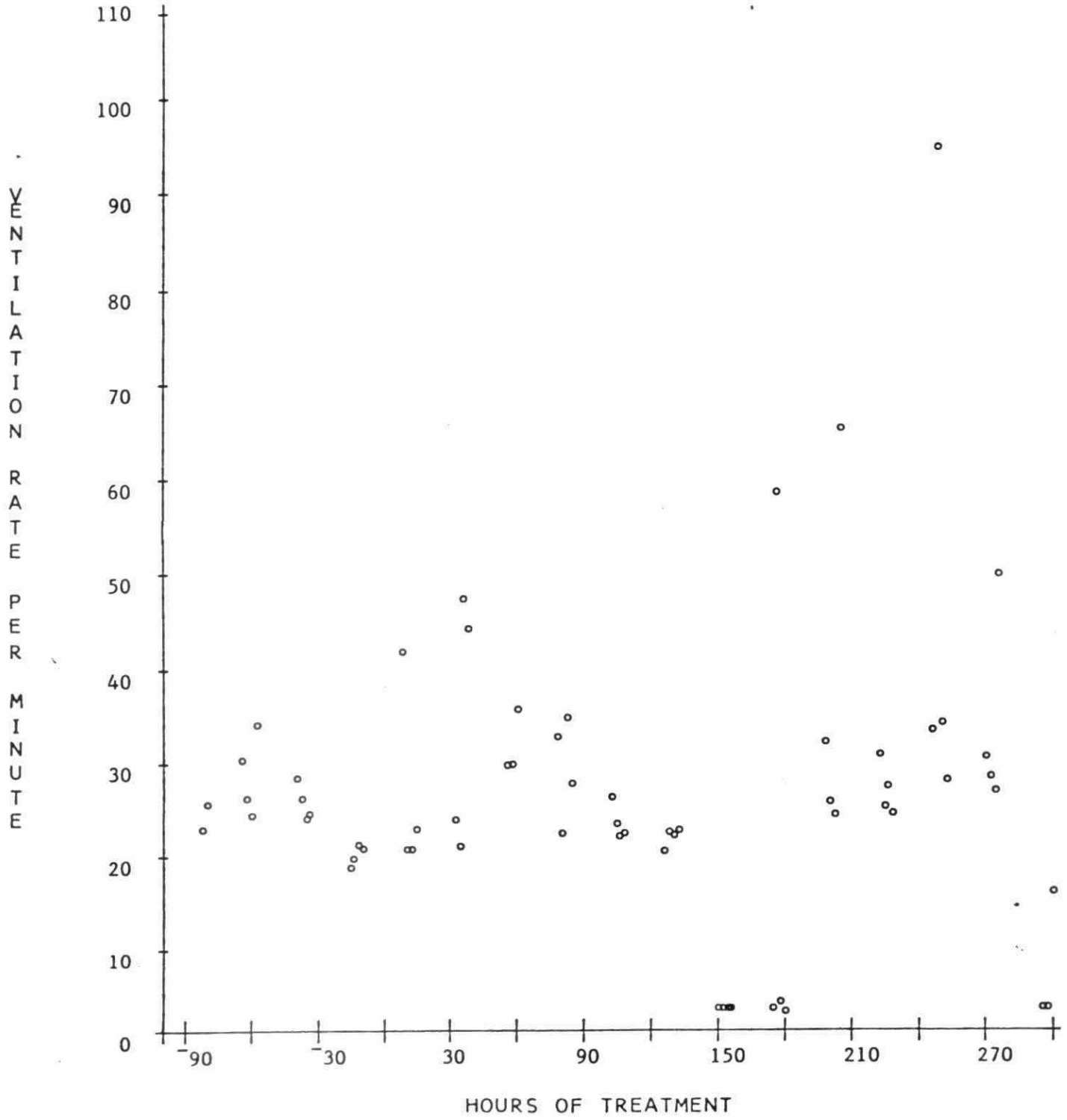
DAY VENTILATION RATE EVERY 2 HOURS FOR FISH 3



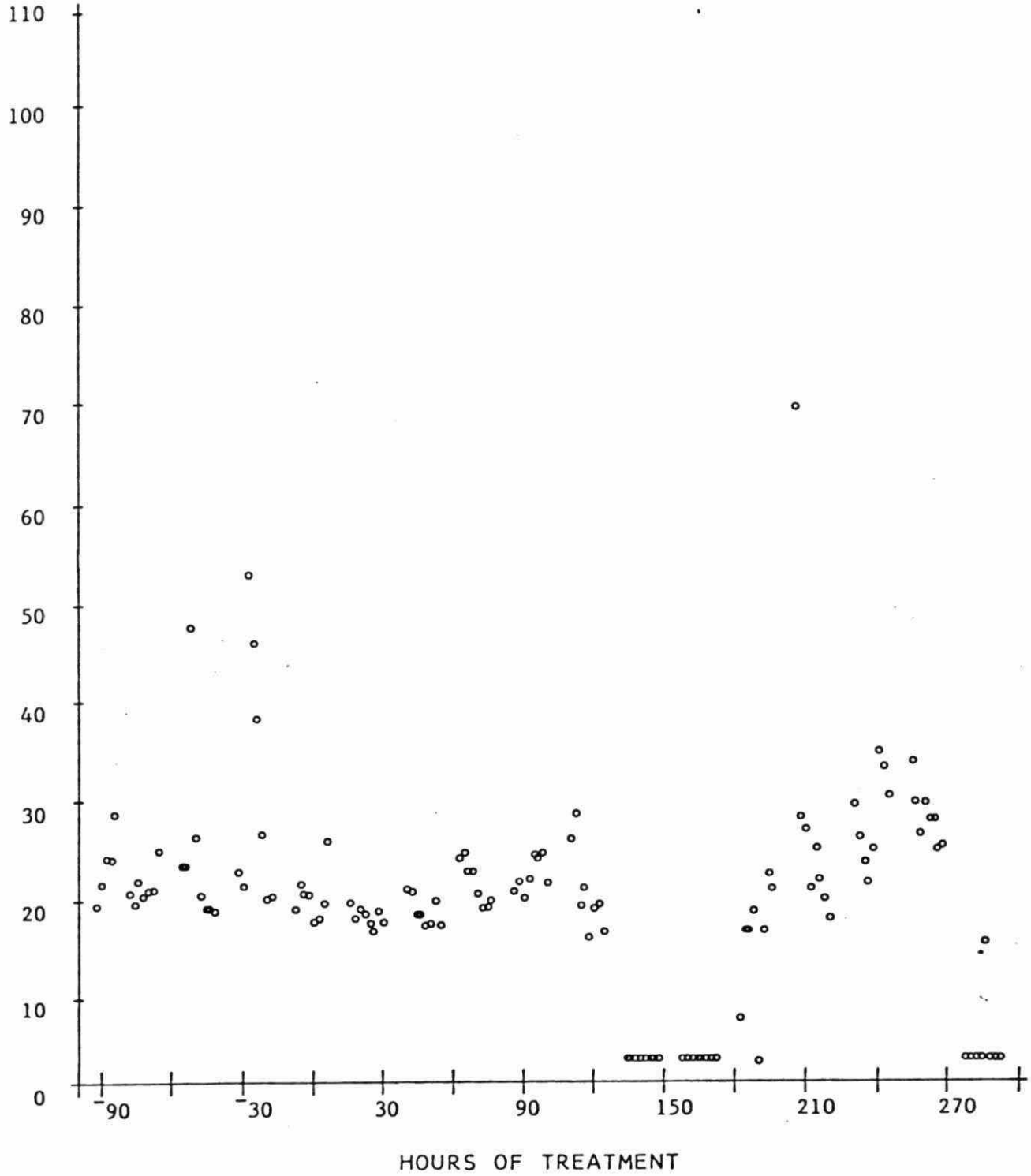
NIGHT VENTILATION RATE EVERY 2 HOURS FOR FISH 3



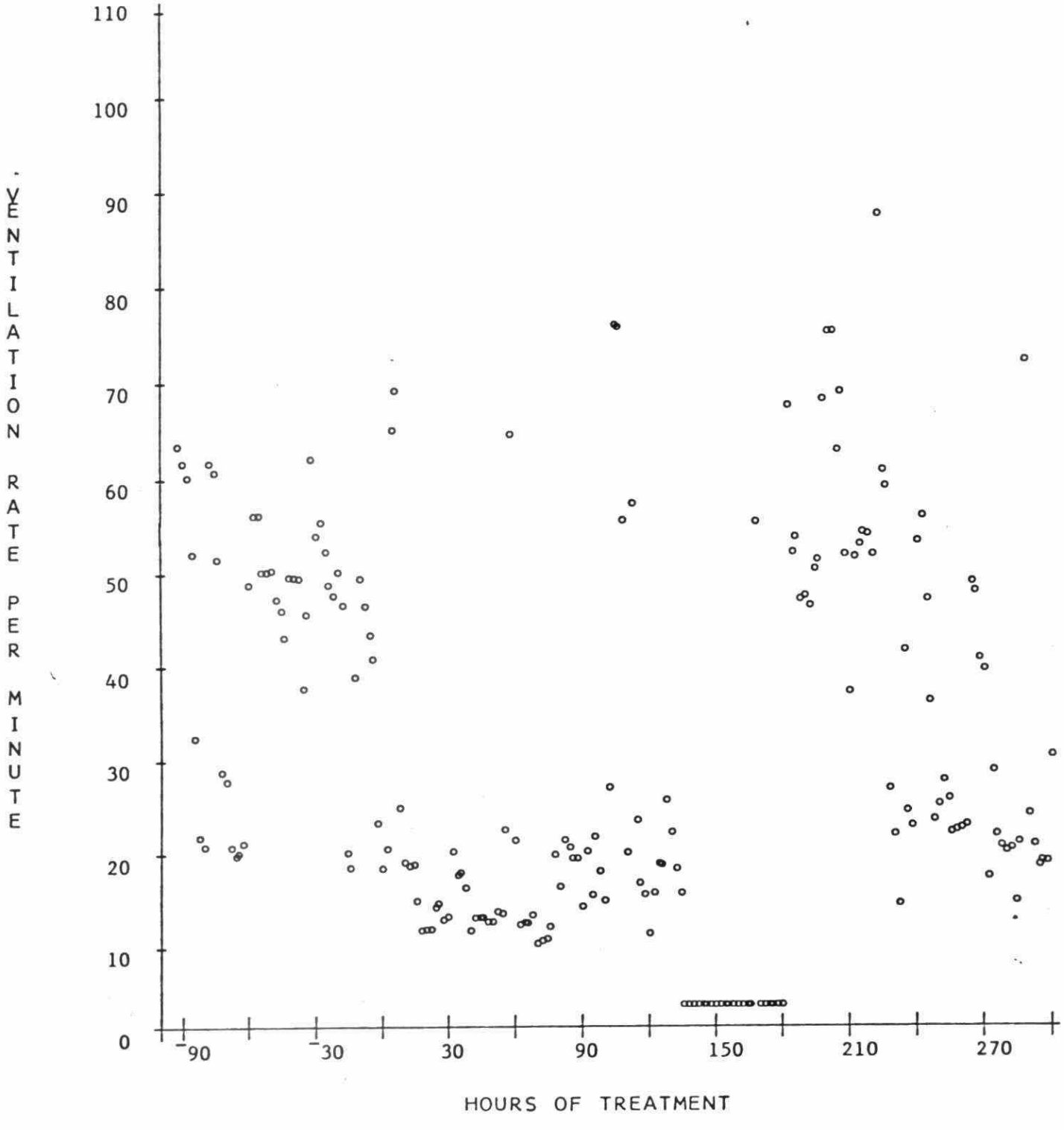
VENTILATION RATE EVERY 2 HOURS FOR FISH 4



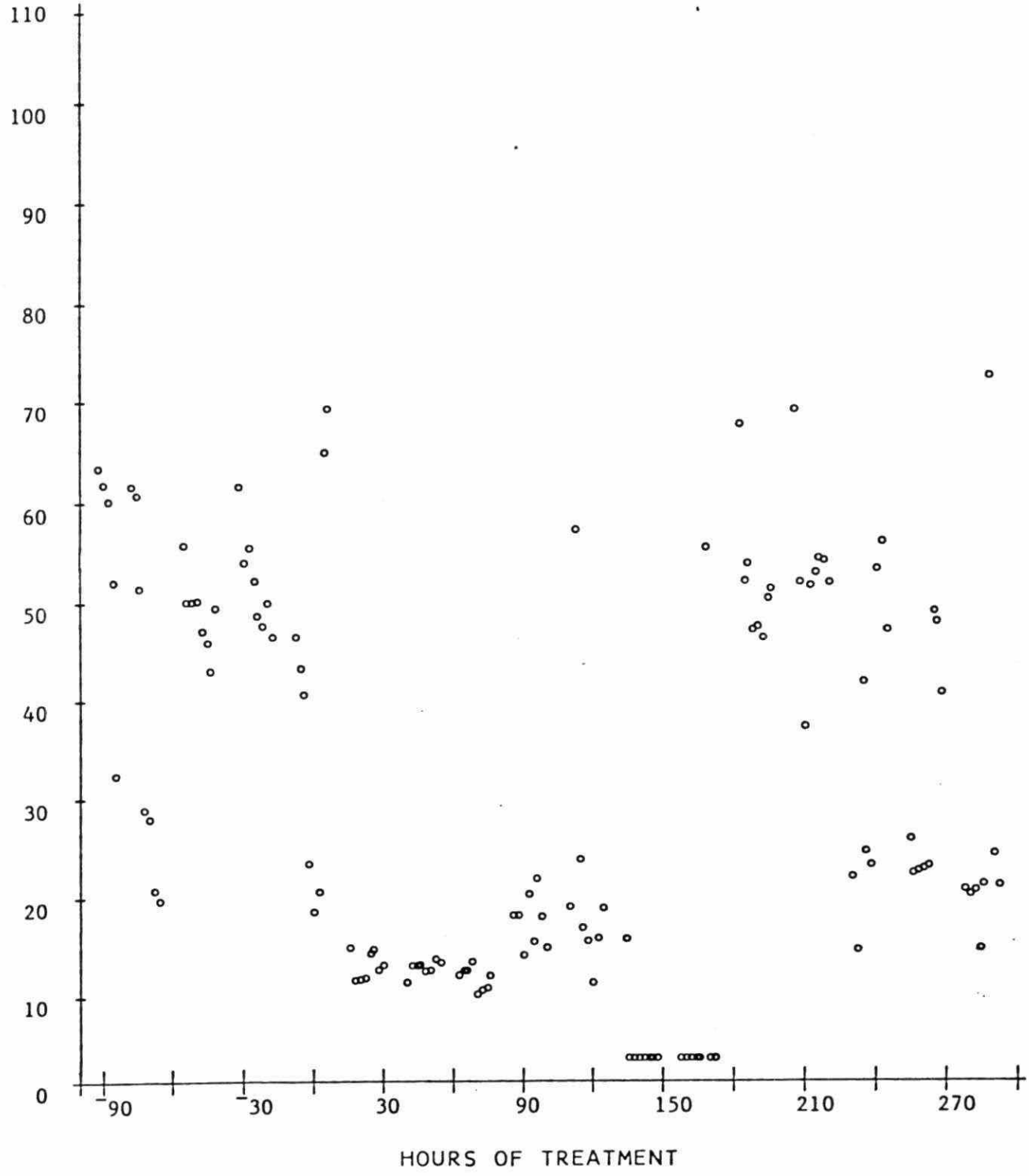
DAY VENTILATION RATE EVERY 2 HOURS FOR FISH 4



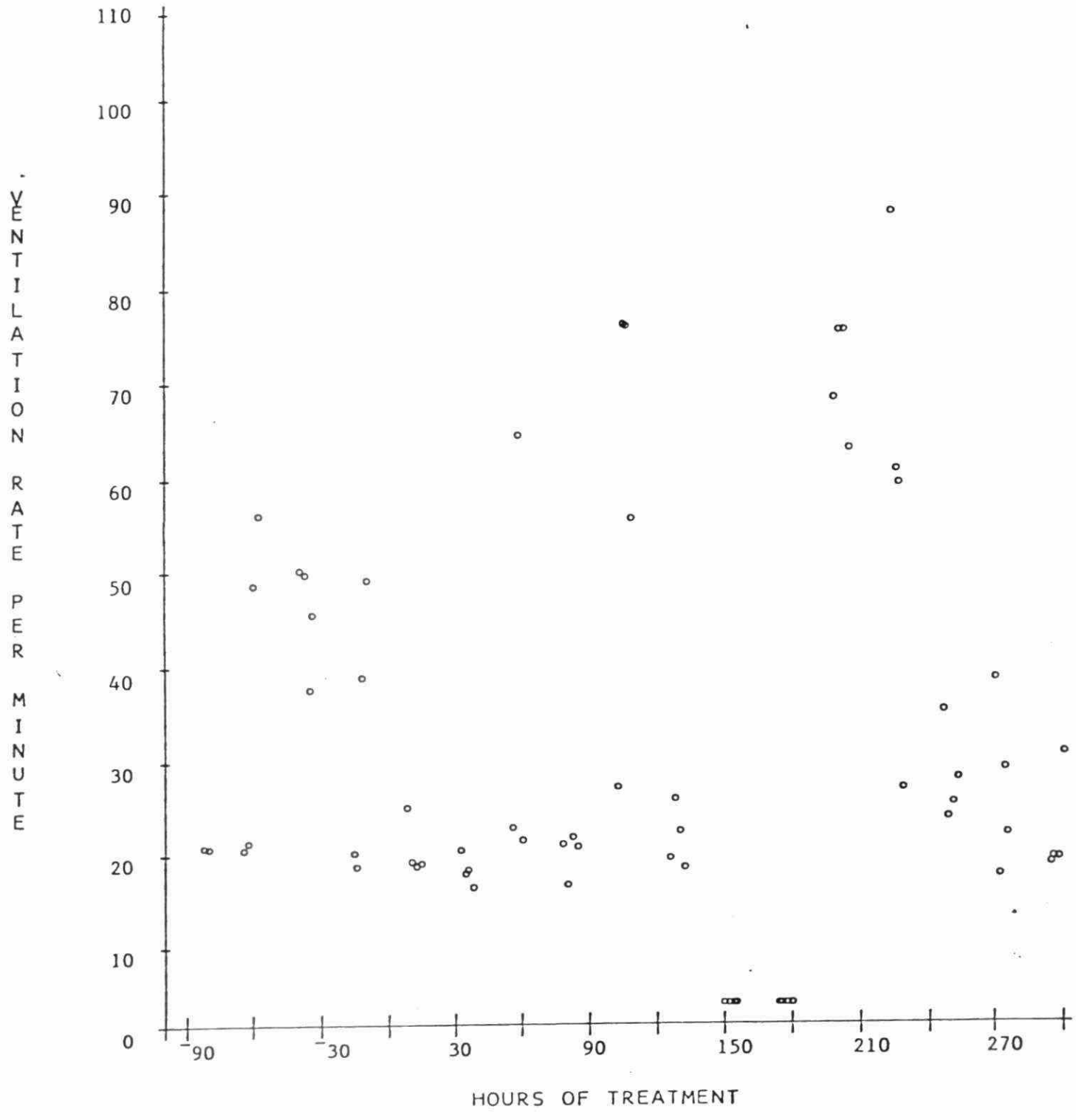
NIGHT VENTILATION RATE EVERY 2 HOURS FOR FISH 4



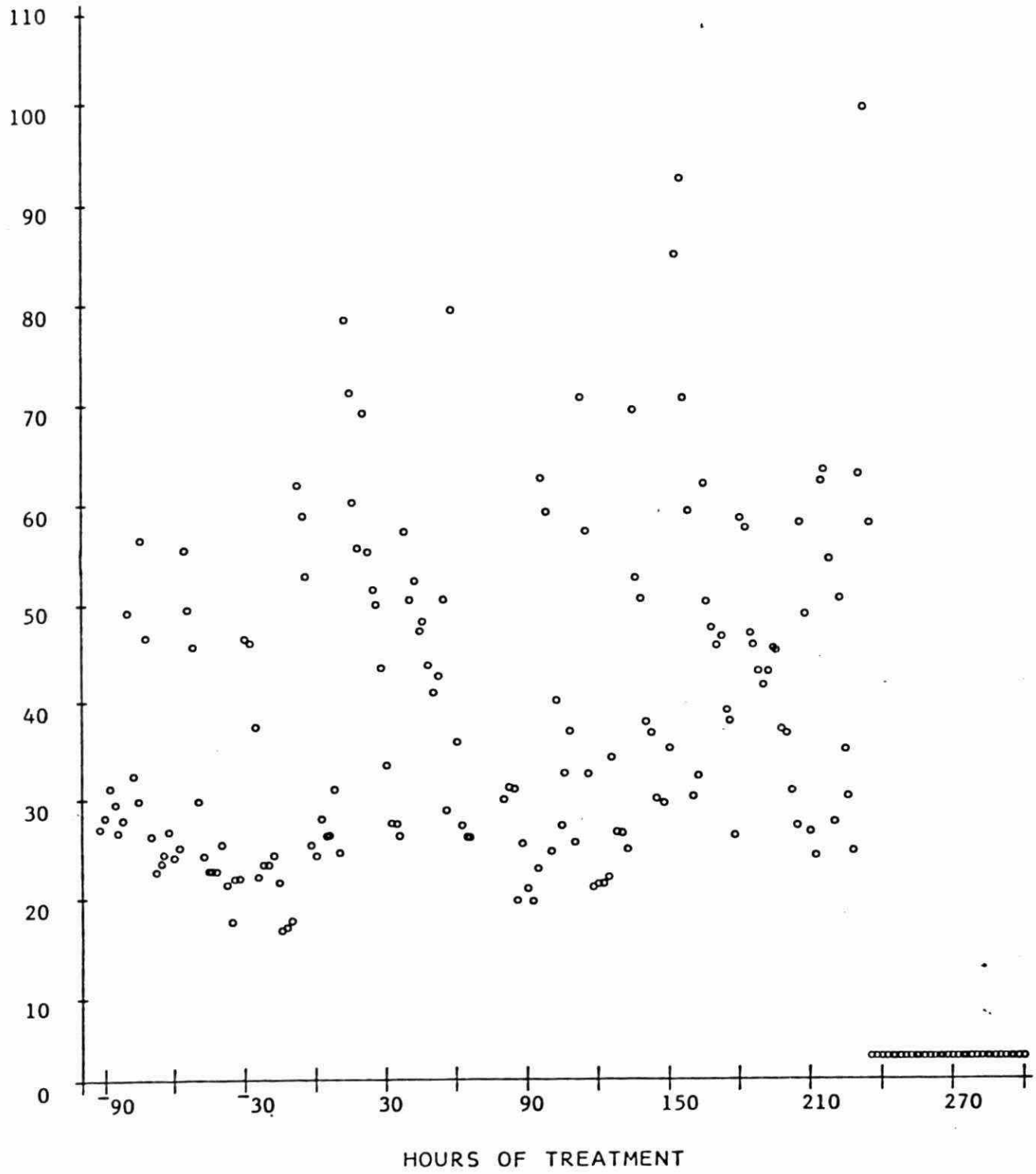
VENTILATION RATE EVERY 2 HOURS FOR FISH 5



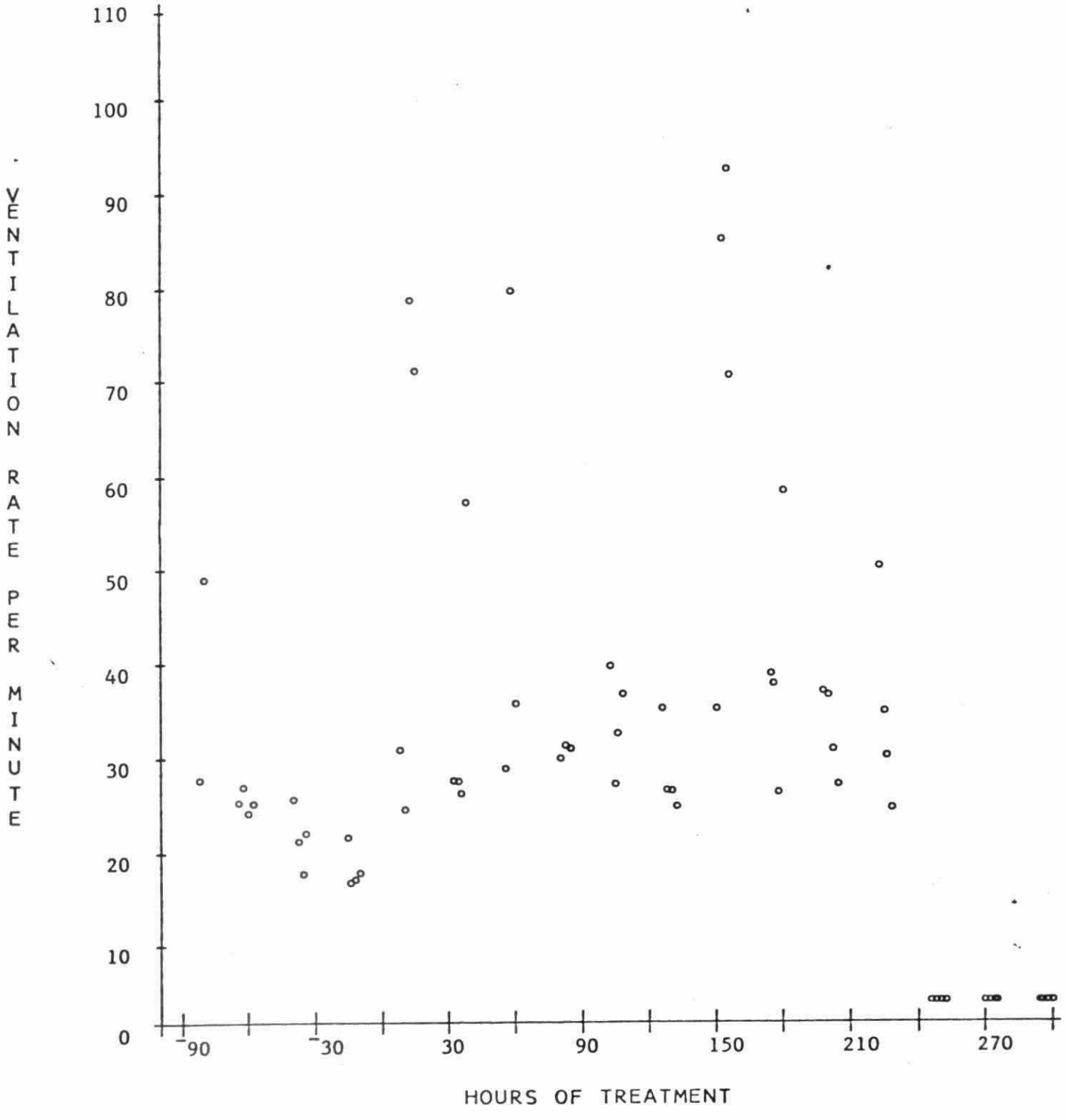
NIGHT VENTILATION RATE EVERY 2 HOURS FOR FISH 5



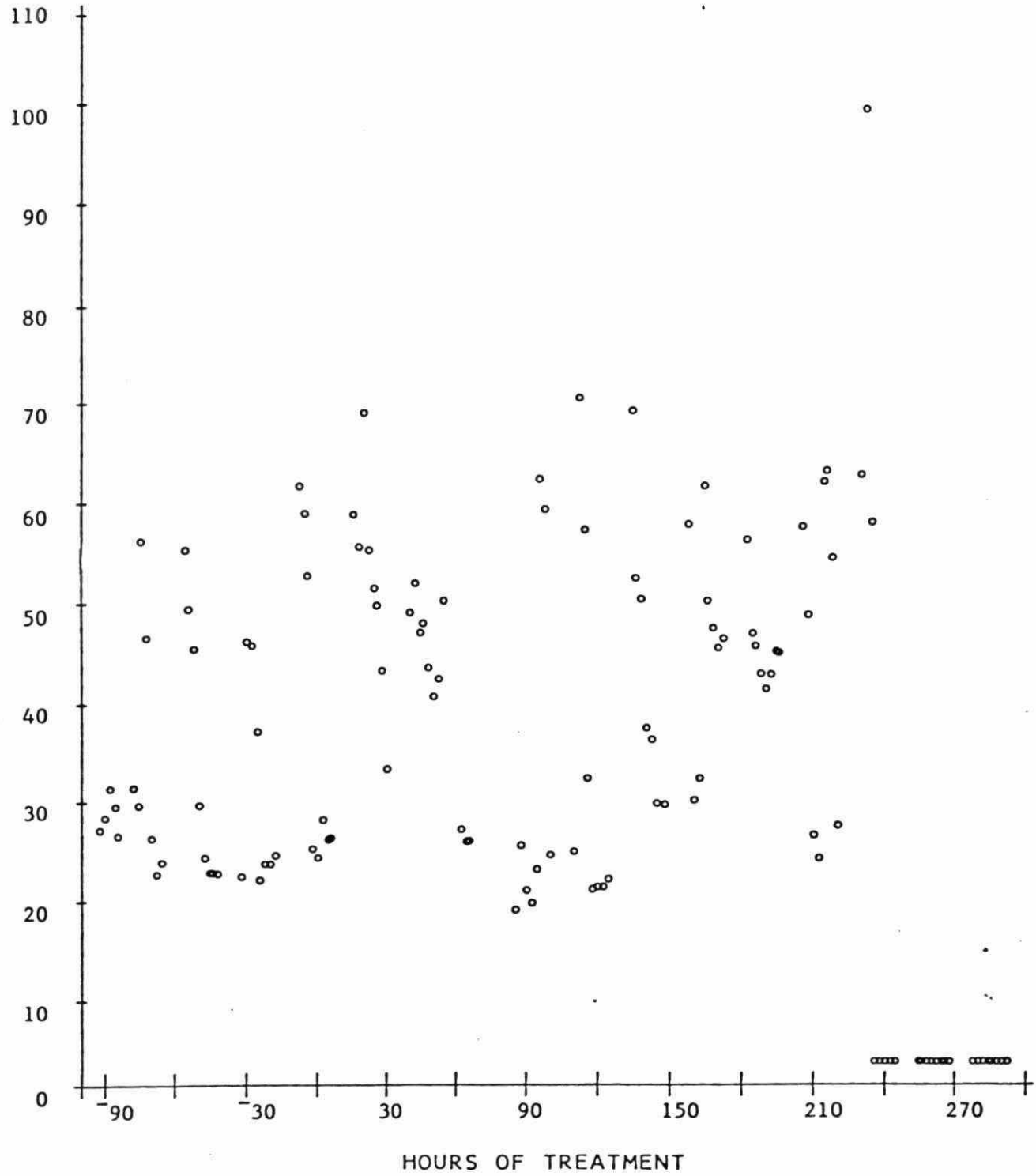
DAY VENTILATION RATE EVERY 2 HOURS FOR FISH 5



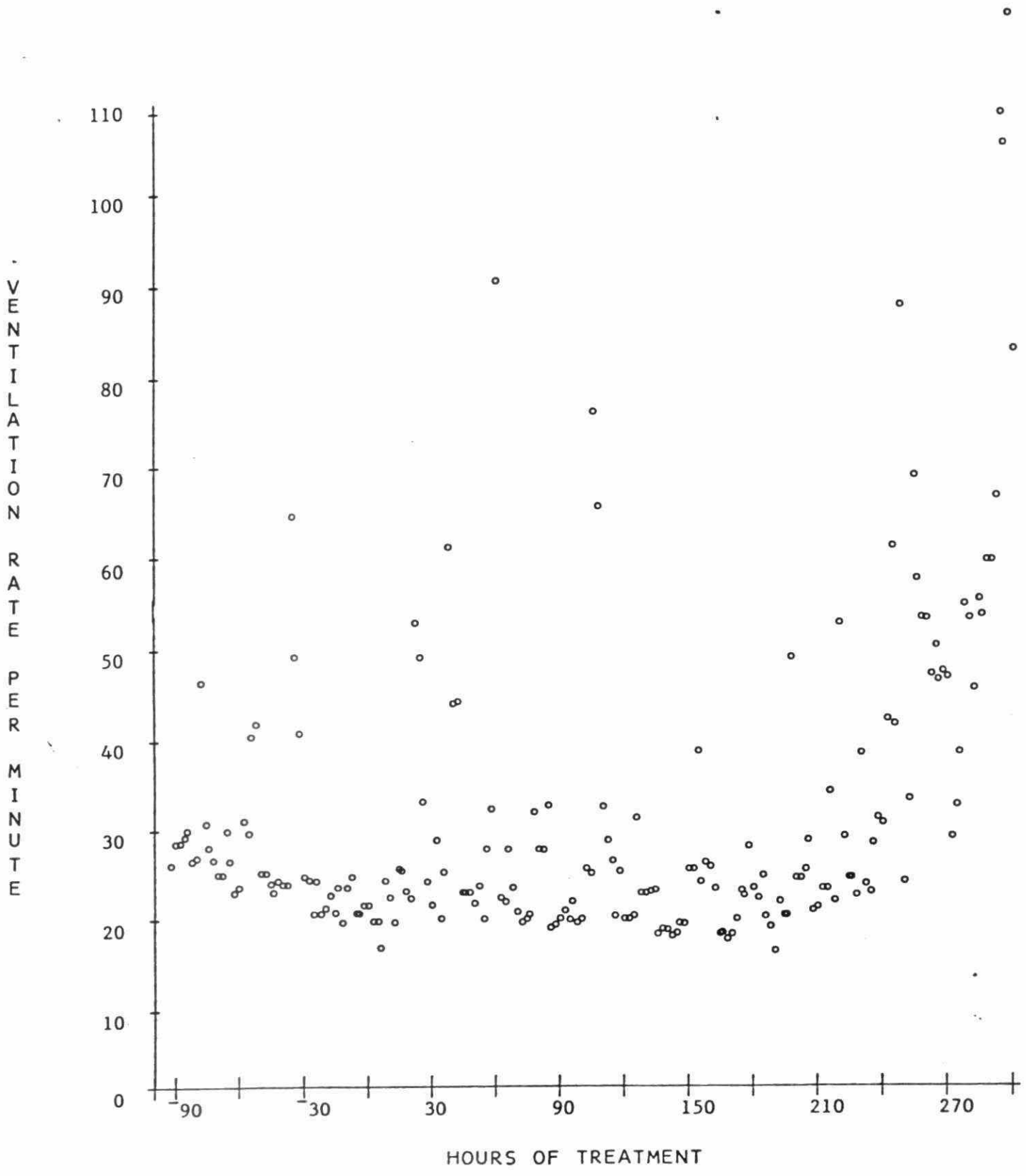
VENTILATION RATE EVERY 2 HOURS FOR FISH 7



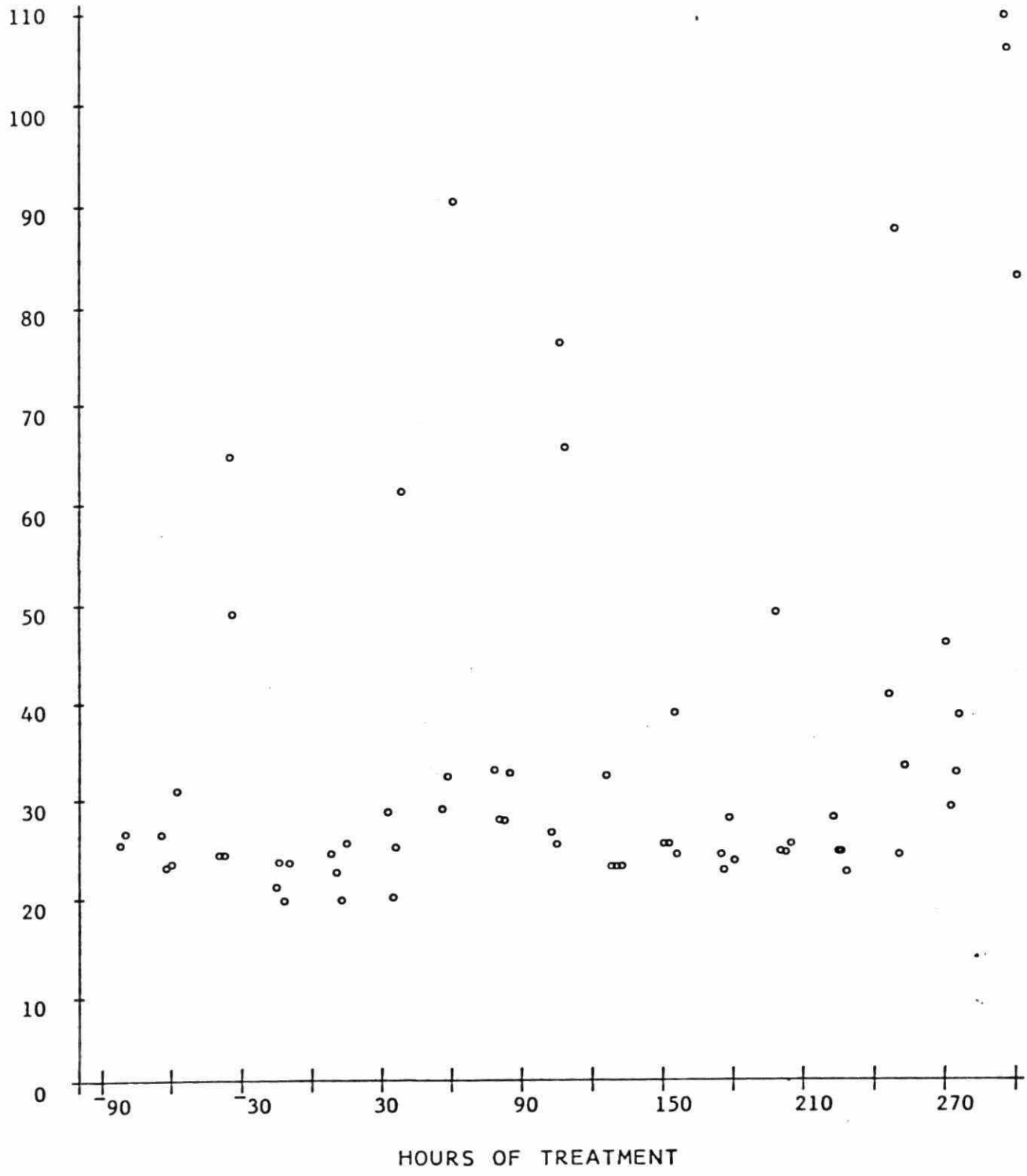
DAY VENTILATION RATE EVERY 2 HOURS FOR FISH 7



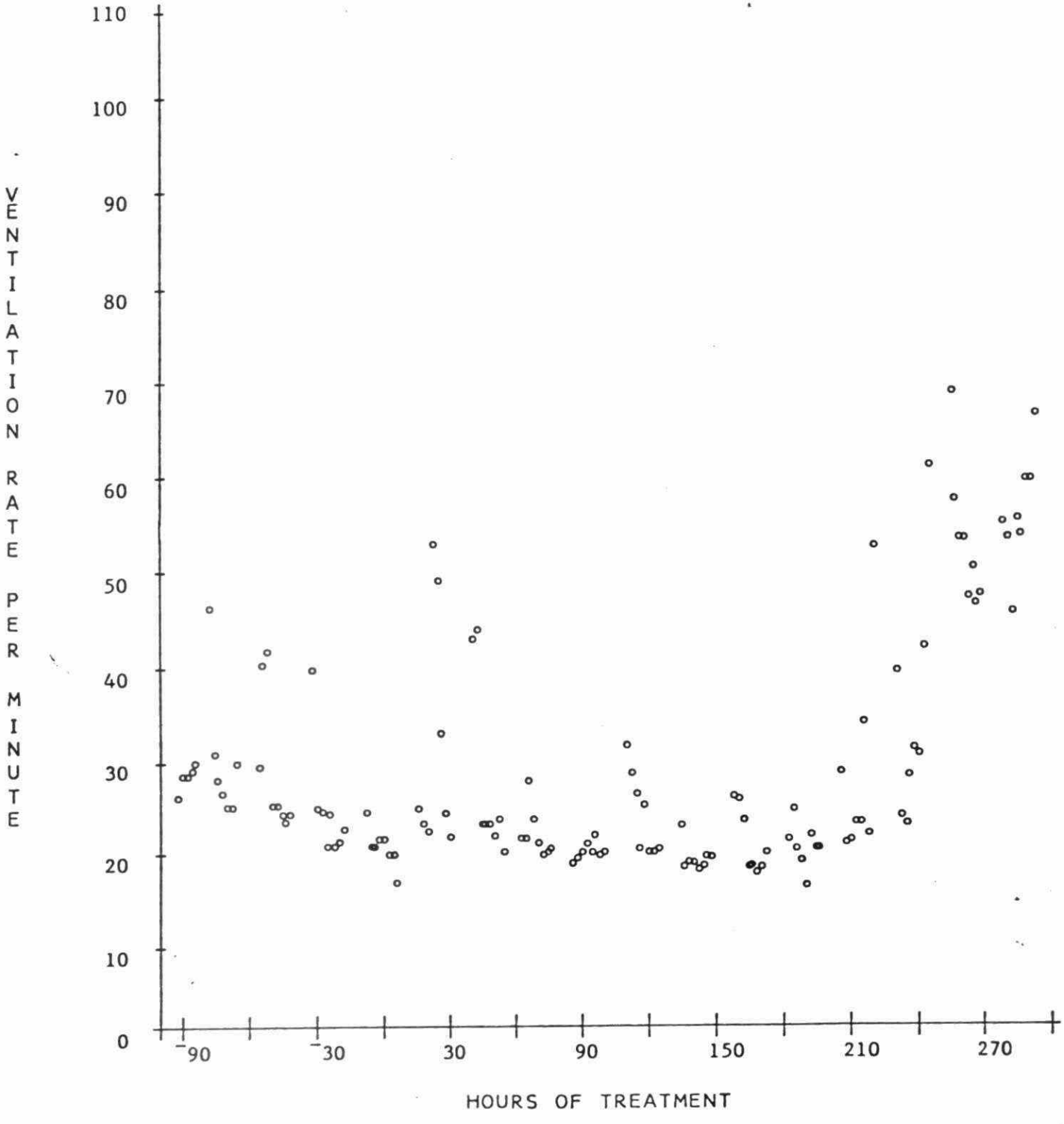
NIGHT VENTILATION RATE EVERY 2 HOURS FOR FISH 7



VENTILATION RATE EVERY 2 HOURS FOR FISH 8



DAY VENTILATION RATE EVERY 2 HOURS FOR FISH 8



NIGHT VENTILATION RATE EVERY 2 HOURS FOR FISH 8